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ORIGINAL ARTICLE

Limited sampling strategies for tacrolimus exposure ($AUC_{0\text{-}24}$) prediction after Prograf® and Advagraf® administration in children and adolescents with liver or kidney transplants

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Summary

To develop limited sampling strategies (LSSs) to predict total tacrolimus exposure ($AUC_{0\text{-}24}$) after the administration of Advagraf® and Prograf® (Astellas Pharma S.A, Madrid, Spain) to pediatric patients with stable liver or kidney transplants. Forty-one pharmacokinetic profiles were obtained after Prograf® and Advagraf® administration. LSSs predicting $AUC_{0\text{-}24}$ were developed by linear regression using three extraction time points. Selection of the most accurate LSS was made based on the r^2, mean error, and mean absolute error. All selected LSSs had higher correlation with $AUC_{0\text{-}24}$ than the correlation found between C_0 and $AUC_{0\text{-}24}$. Best LSS for Prograf® in liver transplants was $C_{0\_1.5\_4}$ ($r^2 = 0.939$) and for kidney transplants $C_{0\_1\_3}$ ($r^2 = 0.925$). For Advagraf®, the best LSS in liver transplants was $C_{0\_1\_2.5}$ ($r^2 = 0.938$) and for kidney transplants was $C_{0\_0.5\_4}$ ($r^2 = 0.931$). Excluding transplant type variable, the best LSS for Prograf® is $C_{0\text{-}1\text{-}3}$ ($r^2 = 0.920$) and the best LSS for Advagraf® was $C_{0\_0.5\_4}$ ($r^2 = 0.926$). Considering transplant type irrespective of the formulation used, the best LSS for liver transplants was $C_{0\_2\_3}$ ($r^2 = 0.913$) and for kidney transplants was $C_{0\_0.5\_4}$ ($r^2 = 0.898$). Best LSS, considering all data together, was $C_{0\_1\_4}$ ($r^2 = 0.898$). We developed several LSSs to predict $AUC_{0\text{-}24}$ for tacrolimus in children and adolescents with kidney or liver transplants after Prograf® and/or Advagraf® treatment.

Introduction

Tacrolimus is the first choice immunosuppressant drug used in solid organ transplantation. Blood concentrations of tacrolimus are related to both efficacy and toxicity, but tacrolimus has a narrow therapeutic window and its pharmacokinetics show high variability among patients. Therefore, therapeutic drug monitoring (TDM) is a routine practice for guiding dose adjustment [1].

Tacrolimus TDM is usually performed by measuring trough levels. However, evidence has shown that total exposure to tacrolimus as reflected by the area under the

curve (AUC) may be a better indicator of the drug's efficacy and toxicity, and this parameter has been acknowledged as the best marker of tacrolimus exposure [2,3]. The exact calculation of AUC requires a large number of samples throughout the dosing interval and is therefore not possible in clinical practice. To overcome this problem, the development of limited sampling strategies (LSSs) has been proposed, to allow for an accurate AUC calculation and a practical sampling schedule (up to three samples in a short period around the administration of tacrolimus) [1]. Several schemes have been proposed to predict the tacrolimus $AUC_{0\text{-}12}$ after the administration of bid formulation (Prograf®) [4,5].

The new formulation of tacrolimus for administration once daily (Advagraf®) has been introduced into clinical practice in the recent years, on the assumption that a once-daily administration would improve the patient's adherence and therefore would prevent organ rejection and graft loss [6]. This new formulation provides very similar total exposure (as reflected by $AUC_{0\text{-}24}$) when compared with the standard formulation [7–9], but its concentration profile is different.

This means the LSS designed to predict the tacrolimus $AUC_{0\text{-}12}$ after Prograf® use could not be valid to estimate the $AUC_{0\text{-}24}$ of tacrolimus after the administration of either Prograf or Advagraf®. The development of LSSs to predict the tacrolimus 24-h exposure, considered relevant for adult patients, could be even more relevant in the pediatric population, whose treatment compliance is estimated to be about four times lower than in the adult population [10].

Purpose

The aim of our study was to develop LSSs predicting tacrolimus $AUC_{0\text{-}24}$ after the administration of Advagraf® and Prograf® in pediatric patients with stable liver or kidney transplants.

Methods

Design

The data for analysis come from two clinical trials. Both investigated tacrolimus disposition after the administration of Prograf® and Advagraf® (both were approved by the hospital's research ethics committee and were performed following good clinical practice guidelines; informed consent was obtained from all patients); the first study was performed on stable pediatric liver transplant recipients [11], and the second study was performed on stable kidney transplant recipients [12].

In both trials, the patients were converted from Prograf® administration to Advagraf® administration. The eligible patients were 12 to 17 years of age (liver transplant) or 4 to 17 years of age (kidney transplant), with stable liver and kidney function, who had been receiving stable doses of Prograf® twice a day for at least 1 month prior to enrollment. After enrollment, the patients maintained this schedule in a supervised way on days 1 to 7 and were then converted to the same daily dose of Advagraf® (once-daily formulation) on days 8 to 14. Twenty-four-hour pharmacokinetic (PK) profiles were obtained on days 7 and 14 for PK analysis of tacrolimus exposure after each formulation administration.

Pharmacokinetic profiles

Serial blood samples to define the concentration-time profiles were collected at the following times: 0 h (pre-intake) and after the first dose at 0.5 h, 1 h, 1.5 h, 2 h, 3 h, 4 h, 6 h, 8 h, 12 h (second dose), 12.5 h, 13 h, 13.5 h, 14 h, 15 h, 16 h, 18 h, 20 h, and 24 h for Prograf® administration (oral dose twice daily). For Advagraf® administration (oral dose once daily), concentration-time profiles were collected at pre-intake and at 0.5 h, 1 h, 1.5 h, 2 h, 2.5 h, 3 h, 4 h, 6 h, 8 h, 12 h, 15 h, and 24 h.

The PK analysis of tacrolimus was first performed on stable patients with Prograf® treatment (Prograf® taken every 12 h). Patients were then switched to Advagraf® every 24 h, and blood extractions for PK analysis were taken after seven doses of this drug.

Assay methods

The concentration levels of tacrolimus in whole blood samples were determined by a homogenous enzyme immunoassay method (DIMENSION®, Siemens Health Care Diagnostic Ltd., Frimley, Camberley, UK). The quantification range was 2–30 ng/ml.

Pharmacokinetic and statistical analysis

The kinetic data analysis was performed following a non-compartmental model. $AUC_{0\text{-}24}$ was calculated by the trapezoidal rule. C_{max} was obtained directly from raw data values.

We used a linear regression analysis to obtain the best time points that correlated with the $AUC_{0\text{-}24}$ for Advagraf® and Prograf®. Based on published LSSs and on their utility in clinical practice, we selected the following time points [13]: For modified release tacrolimus formulation (Advagraf®), the points were C0, C1, C1.5, C2, C2.5, C3, and C4; for Prograf®, the time points were C0, C1, C1.5, C2, C3, and C4. All tested LSSs included C0 and a maximum of three extraction time points. Selection of the most accurate LSS predicting $AUC_{0\text{-}24}$ was performed based on the r^2, mean error (ME), and mean absolute error (MAE),

following Bland and Altman [14]. ME would reflect the bias in the prediction and MAE is an estimation of the precision of the regression equation. The ME was calculated as the mean of the differences between the predicted and observed $AUC_{0\text{-}24}$; the MAE was calculated as the mean of the absolute differences between the predicted and observed $AUC_{0\text{-}24}$; and the MAE% is expressed as a percentage of the observed $AUC_{0\text{-}24}$. For these parameters, a standard deviation and 95% confidence interval were calculated. Visual observation of residuals and homoscedasticity tests were performed. For each group, we first selected the five LSSs with the best r^2; from these five LSSs, we discharged by visual inspection those showing an appreciable bias (ME not centered around zero or with a large 95% CI) and/or with a larger MAE%.

Results

Patient characteristics

Forty-one patients (20 female and 21 male) with stable graft function were included in our study: 20 patients with a liver transplant and 21 patients with a kidney transplant. The patients' primary characteristics are shown in Table 1.

All patients had tacrolimus as the principal immunosuppressive agent. Twenty-three patients were taking prednisolone. Twenty-five patients also received mycophenolate mofetil (MMF). Other drugs taken were clotrimazole (18 patients), deflazacort (two patients), omeprazole (two patients), trimethoprim (one patient), ursodeoxycholic acid (three patients), and valganciclovir (two patients). In addition to the previously referred concomitant drugs, many of the patients were taking food supplements. No changes in concomitant drugs or their dose were performed during the pharmacokinetic study.

Table 1. Primary characteristics of the 41 included patients with liver or kidney transplant.

Hepatic Tx (n = 20)	
Gender (%)	
Male/Female	45/55
Race (%)	
White/Black/Hispanic	85/5/10
Age mean ± SD (range) years	13.90 ± 1.66 (12–17)
Weight: mean ± SD (range)	47.86 ± 9.13 (29.4–67.5)
Height: mean ± SD (range)	155.10 ± 8.26 (139–169)
Time post-transplant mean ± SD (range)	10.30 ± 4.74 (1–16)
Kidney Tx (n = 21)	
Gender (%)	
Male/Female	57/43
Race (%)	
White/Hispanic/Asian/Arabic	80/5/10/5
Age mean ± SD (range) years	12.29 ± 4.17 (4–17)
Weight mean ± SD (range)	42.85 ± 15.42 (15.1–63.8)
Height mean ± SD (range)	143.40 ± 18.16 (105–168)
Time post-transplant mean ± SD (range)	5.39 ± 3.25 (1–12)

SD, standard deviation.

Disposition parameters of Prograf® and Advagraf®

Table 2 shows the primary disposition parameters after Prograf® and Advagraf® formulation administration in both types of transplants. Fig. 1 shows the mean blood concentration of tacrolimus. When patients took Prograf®, C_{max} was achieved with the morning administration in most cases. The disposition parameters are quite similar for both formulations in both transplant types, $AUC_{0\text{-}24}$, C_{max}, and T_{max} for Advagraf® being the only pharmacokinetic values significantly higher in liver patients than in kidney patients, but when $AUC_{0\text{-}24}$ is adjusted by dose and weight, this difference disappears (Figs 2–5).

LSS analysis in liver and kidney transplantation

We analyzed all possible LSSs to determine which was optimal: different extraction times for each formulation in both transplant types; both kidney and liver transplants without taking into account the formulation; both the Advagraf® and Prograf® formulations without taking into account the type of transplant; and for all together. The predictive performance of C0 and the best LSSs are shown in the Supplementary Tables.

We first analyzed all the LSS data separately. The r^2 for C0 and $AUC_{0\text{-}24}$ was quite low in all cases (lower than 0.739), and systematically showed statistical differences in r^2 and MAE% when compared with the best multiple point LSS selected (see Tables 3–6). Statistical differences between the best five preselected multiple point LSSs were usually not detected because their r^2 was high. However, some of the LSSs had an ME that was biased or had a high 95% CI and were therefore discharged, such as in kidney transplantation with extraction points at 0-1-4 or 0-2-3 when the Prograf® formulation was used; or when the Advagraf® formulation was used in liver transplantation with 0-1.5-2.5 extraction points; or in kidney transplantation with 0-1-4 extraction points. A similar process was performed after inspection of MAE%. As an example, the LSS C0-1-2.5 and C0-0.5-4 for Advagraf® in kidney transplantation had a very similar r^2 (0.930 vs. 0.931); however, differences in MAE% were statistically significant (7.522 ± 3.630 vs. 5.100 ± 3.437; paired t-test, $P < 0.05$), and therefore, C0-0.5-4 was selected as the preferred LSS. Thus, we considered that the best LSS for Prograf® when used in patients with a liver transplant was the one that used extraction points at 0, 1.5, and 4 h (r^2 = 0.939). In patients with a kidney transplant, the best LSS had slightly

Table 2. Primary disposition parameters for tacrolimus after administration of Prograf® and Advagraf® formulations in both kidney and liver transplant.

	Prograf® (mean ± SD)				Advagraf® (mean ± SD)			
$AUC_{0\text{-}24}$ N = 41	Kidney Tx	Liver Tx	P	Global	Kidney Tx	Liver Tx	P	Global
$AUC_{0\text{-}24}$ (ng.h/ml)	202.03 ± 42.61	234.92 ± 68.77	0.063	218.21 ± 56.45	178.23 ± 42.61	238.50 ± 68.77	0.002	207.63 ± 63.91
$AUC_{0\text{-}24}$ ((ng.h/ml)/ng/Kg)	2546.0.70 ± 1182.201	2571.980 ± 907.385	0.370	2558.709 ± 1044.060	2248.106 ± 1043.261	2548.624 ± 731.623	0.204	2394.700 ± 906.410
Cl/F (l*h/Kg)	0.48 ± 0.23	0.46 ± 0.19	0.775	0.47 ± 0.21	0.56 ± 0.30	0.45 ± 0.16	0.128	0.51 ± 0.25
Cl/F (l*h)	19.81 ± 11.86	21.35 ± 6.80	0.615	20.56 ± 9.64	25.16 ± 19.17	20.78 ± 7.02	0.342	23.02 ± 14.56
C_{max} (ng/ml)	16.45 ± 5.68	19.52 ± 5.51	0.087	17.95 ± 5.74	13.63 ± 5.03	18.96 ± 5.52	0.002	16.23 ± 5.87
T_{max} (h)	1.5 (1–1.5)	1.5 (1–2)	0.897	1.5 (1–1.5)	2 (1.5–6)	1.5 (1.5–2.38)	0.040	1.5 (1.5–3)

$AUC_{0\text{-}24}$ ((ng.h/ml)/ng/Kg), $AUC_{0\text{-}24}$ adjusted by dose and weight; Cl/F (l*h/Kg), oral clearance adjusted by weight; Cl/F (l*h), oral clearance; C_{max}, maximum concentration; T_{max}, time when maximum concentration is achieved; SD, standard deviation.

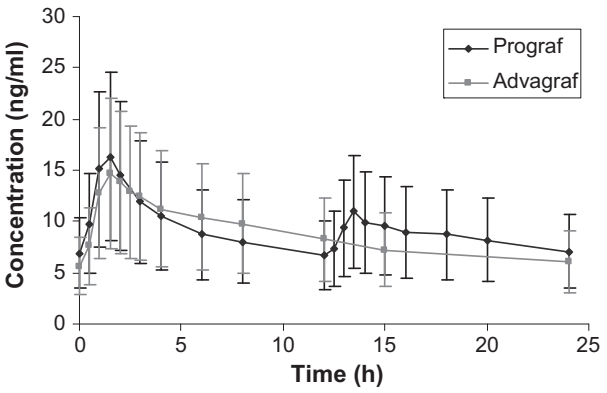

Figure 1 Mean tacrolimus blood concentrations, with 25th and 75th percentile, obtained in children and adolescents with a stable kidney or renal transplant (n = 41).

lower r^2 and was equal to 0.925 when extraction points at 0, 1, and 3 h are used. For the Advagraf® formulation, we obtained an r^2 of 0.938 and an r^2 of 0.931 when administered to liver or kidney transplant patients, respectively. The extraction points used for patients with a liver transplant were at 0, 1, and 2.5 h, and for patients with a kidney transplant, the extraction points were at 0, 0.5, and 4 h. The equations for the selected LSS and for the C0 are presented in Table 3.

Second, we grouped all data by transplant type. We found a low correlation between C0 and $AUC_{0\text{-}24}$ (r^2 = 0.702 in liver transplant and r^2 = 0.569 in kidney transplant). In this case, from all the possible LSSs analyzed with more than one extraction point, we selected the five with the best r^2 obtained for each transplant (Table 4). Again, small differences in r^2 were found between the selected LSSs. Based on the 95% confidence interval representation of the prediction mean error and the MAE%, we selected C0-2-3 as the best LSS for patients with a liver transplant (r^2 = 0.913 and MAE% = 7.48), and for patients with a kidney transplant, the best LSS used points at C0-0.5-4 (r^2 = 0.898 and MAE% = 5.790).

For the third analysis, we grouped all data by formulation and developed the LSS without taking into account the transplanted organ (Table 5). In this analysis, we found a low correlation between C0 and $AUC_{0\text{-}24}$ (r^2 = 0.633 for Prograf® and r^2 = 0.686 for Advagraf®). The best LSS for Prograf® was C0-1-3 (r^2 = 0.920), and for Advagraf®, the best LSS was C0-0.5-4 (r^2 = 0.926).

Finally, we carried out one last analysis of all the collected data with the objective of developing a single LSS for the use in pediatric patients irrespective of the transplant type (kidney or liver) and the formulation used. As with the previous analyses, we found a low correlation between C0 and $AUC_{0\text{-}24}$ and a good fit between the different LSSs with more than one extraction point and their $AUC_{0\text{-}24}$

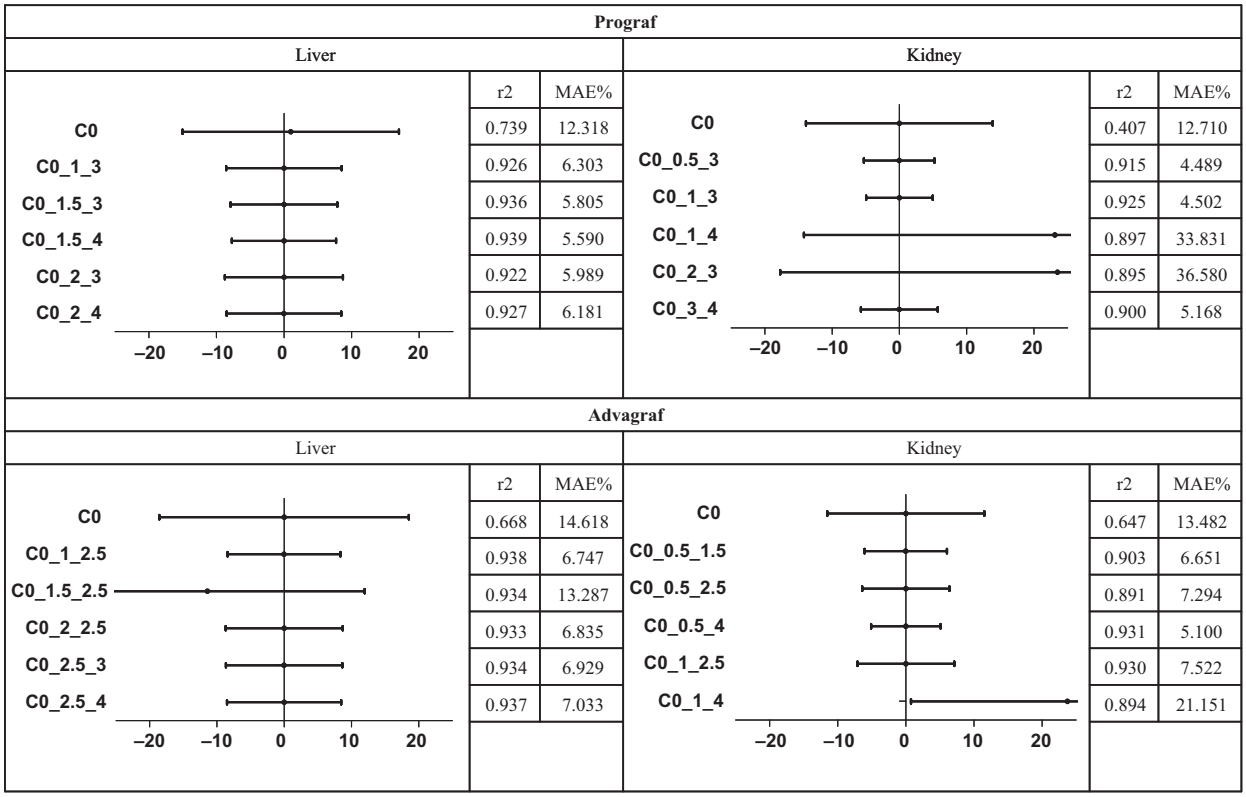

Figure 2 Ninety-five percent confidence interval representation of the mean error, r^2, and MAE% in the predicted $AUC_{0\text{-}24}$, using the best 5 LSSs for Prograf and Advagraf in patients with a liver or kidney transplant.

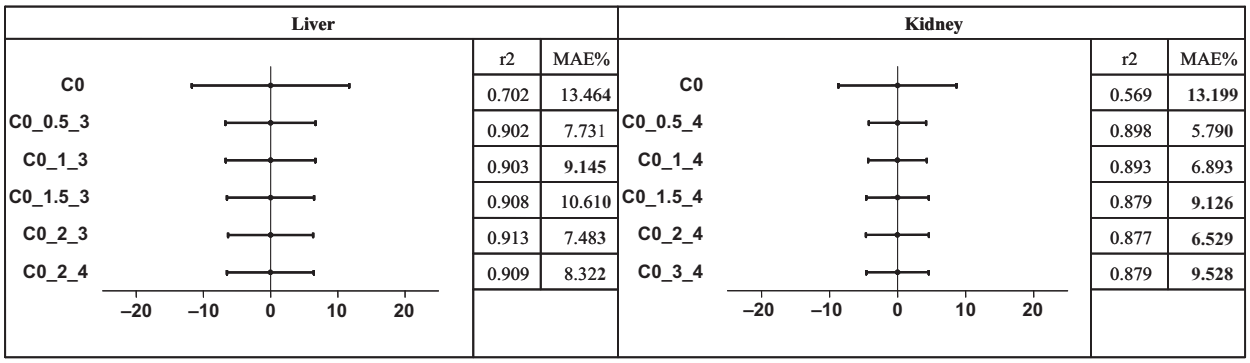

Figure 3 Ninety-five percent confidence interval representation of the mean error, r^2, and MAE% in the predicted $AUC_{0\text{-}24}$ using previous selected LSSs for patients with a liver or kidney transplant.

predictions. All five LSSs had similar r^2 and similar ME and MAE%. We selected C0-1-4 ($r^2 = 0.898$), and its regression equation is shown in Table 6.

Discussion

Studies of patients treated with tacrolimus indicate that, to avoid drug-related rejection and toxicity, it is critical to reach and maintain target blood concentration levels [15,16]. As shown in Table 2, Prograf® and Advagraf® formulations have similar disposition parameters, but each requires a different number of daily administrations and the formulations have different concentration profiles. Therefore, the primary objective of this study was to develop LSSs predicting tacrolimus $AUC_{0\text{-}24}$; as far as we know, no LSS has been developed for the pediatric population that can predict 24-h tacrolimus exposure after Prograf® or Advagraf® administration. This is particularly

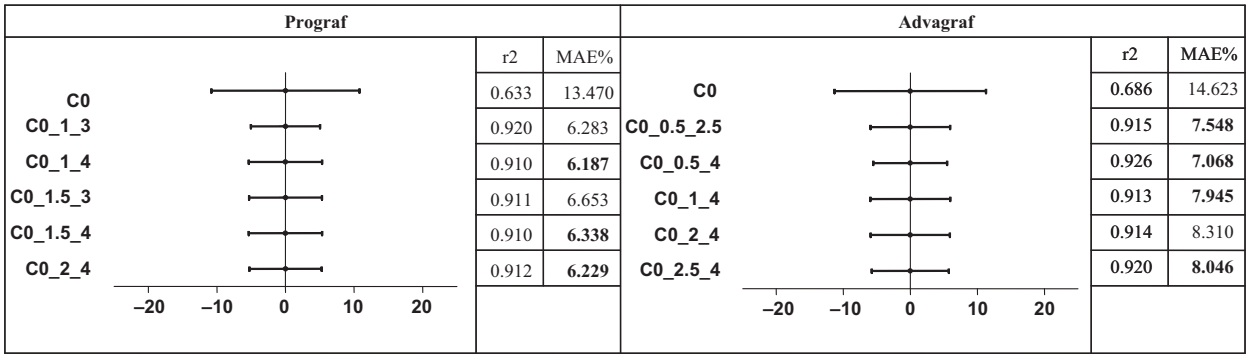

Figure 4 Ninety-five percent confidence interval representation of the mean error, r^2, and MAE% in the predicted $AUC_{0\text{-}24}$, using previously selected LSSs for Prograf and Advagraf formulations.

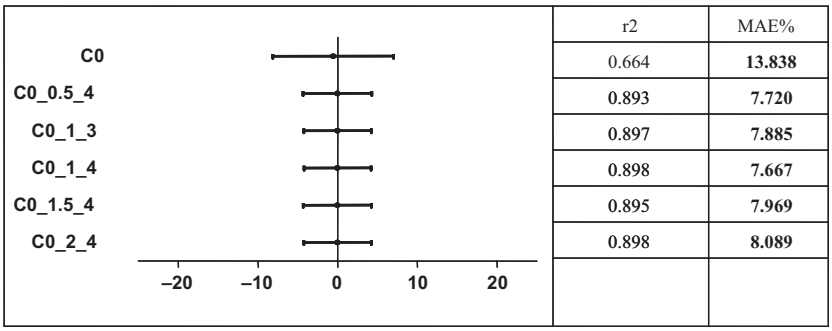

Figure 5 Ninety-five percent confidence interval representation of the mean error, r^2, and MAE% in the predicted $AUC_{0\text{-}24}$, using previously selected LSSs for all data.

important for Advagraf® because this formulation is replacing Prograf® in clinical practice.

Some older studies predicting tacrolimus $AUC_{0\text{-}12}$ have shown a very high r^2 obtained with only C0 [16,17], but prediction of $AUC_{0\text{-}12}$ by means of LSS after Prograf® administration is better than that obtained with C0 [18–22]. The use of LSSs to calculate $AUC_{0\text{-}24}$ after Prograf® administration is appealing because it indicates the total daily tacrolimus exposure instead of only the tacrolimus exposure after the morning dose.

In addition, the use of $AUC_{0\text{-}24}$ would be useful not only for TDM, but for research purposes. The relationship between a range of AUC values and clinical outcomes is not fully clear at this time, but a consensus document [3] concluded that an $AUC_{0\text{-}12}$ target between 150 and 200 ng/ml*h^{-1} is probably appropriate, although evidence is limited. Also, a recent pooled analysis found no relationship between C0 and acute rejection in renal transplant [23]. This result emphasizes the need to develop tools to better predict total daily drug exposure that can improve tacrolimus dosing and increase the efficacy and safety of its use.

Similar to other authors [24], we found a low correlation between C0 and $AUC_{0\text{-}24}$ for both Prograf® and Advagraf® that is parallel with higher MAE% in all cases in comparison with those obtained with the different LSSs proposed (Tables 3–6). Also, the percentage of curves with an absolute error in the predicted AUC above 15% or 20% is higher when using C0 in comparison with the selected LSS (Table 7).

Several analyses were carried out to find the best LSS to predict $AUC_{0\text{-}24}$ in patients treated with tacrolimus (either Prograf® or Advagraf® administration) who had a previous kidney or liver transplants. The r^2, ME, and MAE% were favorable in most of the LSSs selected, with r^2 above 0.90 and MAE% lower than 10%. As previously noted, no LSSs to predict $AUC_{0\text{-}24}$ after Prograf® administration have been published. Several approaches using Bayesian and multilinear regression (MLR) are in the literature to predict $AUC_{0\text{-}24}$ after Advagraf® administration in adults, and all showed positive results. Woillard *et al.* [25] developed a single population PK model in adult kidney transplant recipients that in turn was used to develop a Bayesian estimator able to predict tacrolimus interdose AUC following Advagraf® or Prograf® administration, using concentrations measured at 0 h, 1 h, and 3 h postdose. A similar approach was used by Benkali *et al.* [26],

Table 3. Best 5 LSSs and C0 LSSs developed for each transplant type and each tacrolimus formulation.

Prograf						
	Liver	MAE% (n = 20)				
Predictors	r^2	Mean	Min	Max	SD	Equations
C0	0.739	12.30819	0.690384	40.11089	8.240373	$Y = 14.483C_0 + 133.242$
C0-C1-C3	0.926	6.303042	0.233525	20.58052	3.677639	$Y = 4.503C_0 + 1.483C_1 + 9.119C_3 + 61.691$
C0-C1.5-C3	0.936	5.80471	0.311822	18.61163	3.57721	$Y = 4.908C_0 + 2.638C_{1.5} + 7.249C_3 + 59.054$
C0-C1.5-C4	0.939	5.589516	0.126788	18.11334	3.426092	$Y = 4.669C_0 + 3.597C_{1.5} + 8.748C_4 + 38.578$
C0-C2-C3	0.922	5.988532	0.055517	19.08913	4.321438	$Y = 5.076C_0 + 2.323C_2 + 7.246C_3 + 69.449$
C0-C2-C4	0.927	6.180506	0.127676	21.19897	3.516408	$Y = 4.933C_0 + 3.841C_2 + 7.88C_4 + 51.707$
	Kidney	MAE% (n = 21)				
Predictors	r^2	Mean	Min	Max	SD	Equations
C0	0.407	12.71018	0.017604	28.84895	5.426805	$Y = 19.129C_0 + 79.233$
C0-C0.5-C3	0.915	4.488515	0.162394	10.76989	3.189111	$Y = 7.974C_0 + 1.836C_{0.5} + 10.149C_3 + 20.461$
C0-C1-C3	0.925	4.501561	0.624655	9.699175	2.741723	$Y = 7.923C_0 + 2.017C_1 + 9.281C_3 + 19.874$
C0-C1-C4	0.897	33.83141	5.398065	98.3974	18.73616	$Y = 6.696C_0 + 1.603C_1 + 12.069C_4 + 18.507$
C0-C2-C3	0.895	36.58041	5.503578	115.107	20.56869	$Y = 8.466C_0 + 1.07C_2 + 9.686C_3 + 26.194$
C0-C3-C4	0.900	5.168134	0.956513	10.06956	2.652792	$Y = 7.348C_0 + 7.288C_3 + 5.65C_4 + 19.085$
Advagraf						
	Liver	MAE% (n = 20)				
Predictors	r^2	Mean	Min	Max	SD	Equations
C0	0.668	14.61761	0.264628	67.58868	8.106019	$Y = 14.449C_0 + 136.06$
C0-C1-C2.5	0.938	6.747053	0.045294	24.51441	3.937699	$Y = 12.243C_0 - 1.593C_1 + 8.06C_{2.5} + 54.394$
C0-C1.5-C2.5	0.934	13.28696	2.004326	42.48581	7.162931	$Y = 11.579C_0 - 0.416C_{1.5} + 7.337C_{2.5} + 52.257$
C0-C2-C2.5	0.933	6.835424	1.701747	23.9125	3.55067	$Y = 11.581C_0 - 0.075C_2 + 7.094C_{2.5} + 49.715$
C0-C2.5-C3	0.934	6.929021	2.578974	25.05787	3.498057	$Y = 11.017C_0 + 5.855C_{2.5} + 1.306C_3 + 51.076$
C0-C2.5-C4	0.937	7.033316	2.219183	24.09748	3.082151	$Y = 11.326C_0 + 5.395C_{2.5} + 2.296C_4 + 45.048$
	Kidney	MAE% (n = 21)				
Predictors	r^2	Mean	Min	Max	SD	Equations
C0	0.647	13.4815	3.041935	36.78929	8.158362	$Y = 20.91C_0 + 74.484$
C0-C0.5-C1.5	0.903	6.650506	1.151705	16.93379	3.850905	$Y = 8.209C_0 + 6.367C_{0.5} + 3.482C_{1.5} + 54.19$
C0-C0.5-C2.5	0.891	7.294103	0.852485	15.73897	3.784086	$Y = 8.953C_0 + 7.03C_{0.5} + 3.375C_{2.5} + 51.818$
C0-C0.5-C4	0.931	5.100428	0.338447	16.45873	3.437357	$Y = 8.025C_0 + 5.294C_{0.5} + 8.428C_4 + 27.97$
C0-C1-C2.5	0.930	7.521946	0.789649	21.25963	3.625576	$Y = 13.162C_0 + 4.546C_1 + 1.774C_{2.5} + 45.992$
C0-C1-C4	0.894	21.15137	0.532591	107.6157	16.93722	$Y = 11.696C_0 + 2.798C_1 + 6.947C_4 + 28.271$

using sampling times at 0 h, 1 h, and 3 h after administration. Niioka *et al.* [27] describe an LSS by MLR using only C0 and C12 (r^2 = 0.9221 and MAE% = 7.6%) (Table 8).

The r^2 of our LSS, developed to predict $AUC_{0\text{-}24}$, is slightly lower than other proposed LSSs. However, we believe that these differences in r^2 are minor. Also, the MAE% we obtained is similar to that described in the literature and low enough to be considered reasonably accurate and precise.

Our study has some limitations. First, MLR considers the various time points as independent variables, when in fact they are not, and sampling times must be strictly respected for their valid use; despite these drawbacks, MLR methods have been frequently used in the literature, and they have been shown to be accurate in predicting the "true" AUC, primarily in adults. Second, the number of patients is small; however, the extensive sampling times over the 24-h period after the administration of both formulations allow an appropriate selection of the time points to include in the LSS and encourage a precise calculation of $AUC_{0\text{-}24}$, with good internal precision and accuracy. In fact, if we consider the number of cases with absolute deviations higher than 15–20% in the predicted AUC in relation to the real AUC, the LSSs selected are acceptable, and the number of incorrect predictions

Table 4. Best 5 LSSs and C0 LSSs developed for each transplant type, using data from both tacrolimus formulations.

	Liver	MAE% (n = 40)				
Predictors	r^2	Mean	Min	Max	SD	Equations
C0	0.702	13.46438	0.550444	65.9395	7.927839	$Y = 14.466C_0 + 134.148$
C0-C0.5-C3	0.902	7.731204	0.035125	39.37117	4.020959	$Y = 6.931C_0 + 1.774C_{0.5} + 6.895C_3 + 73.004$
C0-C1-C3	0.903	9.1447	0.967673	36.75901	4.892751	$Y = 7.664C_0 + 1.283C_1 + 6.685C_3 + 67.306$
C0-C1.5-C3	0.908	10.60993	0.084127	45.44014	6.912615	$Y = 7.903C_0 + 1.752C_{1.5} + 6.122C_3 + 62.747$
C0-C2-C3	0.913	7.482981	0.103671	38.41265	4.532379	$Y = 8.062C_0 + 2.850C_2 + 4.763C_3 + 66.882$
C0-C2-C4	0.909	8.322053	0.344451	38.1759	4.807862	$Y = 8.873C_0 + 3.965C_2 + 4.515C_4 + 55.555$
	Kidney	MAE% (n = 42)				
Predictors	r^2	Mean	Min	Max	SD	Equations
C0	0.569	13.19928	0.504052	36.85074	6.786441	$Y = 19.421C_0 + 79.612$
C0-C0.5-C4	0.898	5.790338	0.206466	16.71073	3.691557	$Y = 7.918C_0 + 2.264C_{0.5} + 10.995C_4 + 23.532$
C0-C1-C4	0.893	6.893472	0.027159	17.12601	4.817117	$Y = 8.810C_0 + 1.822C_1 + 10.074C_4 + 23.100$
C0-C1.5-C4	0.879	9.126351	0.404333	19.91848	4.27705	$Y = 9.614C_0 + 0.718C_{1.5} + 17.231C_4 + 20.771$
C0-C2-C4	0.877	6.528577	0.098347	15.74938	3.422307	$Y = 9.470C_0 - 0.633C_2 + 13.820C_4 + 14.749$
C0-C3-C4	0.879	9.528217	0.4761	22.93833	4.657284	$Y = 9.197C_0 - 1.860C_3 + 14.982C_4 + 16.869$

Table 5. Best 5 LSSs and C0 LSSs developed for Prograf® and Advagraf® using data from both transplant types.

	Prograf	MAE% (n = 41)				
Predictors	r^2	Mean	Min	Max	SD	Equations
C0	0.633	13.47038	0.882936	33.94488	8.045677	$Y = 15.420C_0 + 114.072$
C0-C1-C3	0.920	6.282808	0.102757	17.33522	3.609771	$Y = 4.496C_0 + 1.867C_1 + 9.507C_3 + 45.945$
C0-C1-C4	0.910	6.187044	0.087025	23.40342	3.998697	$Y = 4.619C_0 + 2.174C_1 + 11.820C_4 + 30.250$
C0-C1.5-C3	0.911	6.652557	0.131056	16.01466	3.385605	$Y = 5.029C_0 + 1.592C_{1.5} + 8.796C_3 + 53.087$
C0-C1.5-C4	0.910	6.338316	0.896596	22.7756	3.397171	$Y = 5.205C_0 + 2.231C_{1.5} + 10.372C_4 + 37.918$
C0-C2-C4	0.912	6.228678	0.310612	20.80034	3.321274	$Y = 5.176C_0 + 3.356C_2 + 8.675C_4 + 43.813$
	Advagraf	MAE% (n = 41)				
	r^2	Mean	Min	Max	SD	Equations
C0	0.686	14.62333	0.398848	53.55321	9.575361	$Y = 17.005C_0 + 105.602$
C0-C0.5-C2.5	0.915	7.547901	0.088651	22.04498	4.35629	$Y = 9.188C_0 + 3.975C_{0.5} + 5.391C_{2.5} + 51.663$
C0-C0.5-C4	0.926	7.068407	0.180006	26.63747	4.086203	$Y = 7.970C_0 + 4.179C_{0.5} + 7.679C_4 + 41.490$
C0-C1-C4	0.913	7.945123	0.48714	30.69494	4.362738	$Y = 10.637C_0 + 1.434C_1 + 7.634C_4 + 40.462$
C0-C2-C4	0.914	8.309679	0.239245	29.3464	4.224294	$Y = 10.965C_0 + 1.476C_2 + 7.204C_4 + 41.583$
C0-C2.5-C4	0.920	8.045909	0.262406	27.63689	4.109036	$Y = 11.340C_0 + 2.380C_{2.5} + 5.845C_4 + 43.564$

between the selected LSSs does not reach statistical significance, although there is a tendency against the "global" LSS. Third, an external validation would be advantageous; unfortunately, this validation would require an extensive sampling in a new cohort, which is costly and difficult to perform in these types of patients. Fourth, the range of children's ages is somewhat different in each transplant type (almost all patients with a liver transplant are adolescents, whereas the age range is wider in patients with kidney transplant). Although this could introduce heterogeneity in LSSs for the use in mixed populations, the sample gains in representativeness and the MAE% do not appear to be significantly affected if we compare the data in Tables 5–7 with the data in Table 3. As our two studies only include patients from 4 to 17 years old, our results cannot be extrapolated to younger children. The concomitant drugs, as proton pump inhibitors, can also change tacrolimus PK, but we think that these effects should not be appreciated in this kind of analysis (the relationship between punctual drug levels and AUC0-24) and especially in stable patients, that were well controlled and inside the therapeutic window.

Table 6. Best 5 LSSs and C0 LSSs developed when using all data in the same model.

Predictors	r^2	MAE% (n = 82) Mean	Min	Max	SD	Equations
C0	0.664	13.83787	0.367477	54.67136	8.722971	$Y = 16.230C_0 + 109.427$
C0-C0.5-C4	0.893	7.72037	0.001259	30.5734	4.405717	$Y = 7.746C_0 + 2.803C_{0.5} + 8.820C_4 + 43.613$
C0-C1-C3	0.897	7.885038	0.087577	32.1104	4.478814	$Y = 8.149C_0 + 2.195C_1 + 6.292C_3 + 53.077$
C0-C1-C4	0.898	7.66713	0.352815	32.0049	4.206705	$Y = 8.772C_0 + 2.297C_1 + 7.926C_4 + 39.179$
C0-C1.5-C4	0.895	7.969032	0.036839	34.40953	3.940411	$Y = 9.343C_0 + 2.106C_{1.5} + 7.303C_4 + 41.992$
C0-C2-C4	0.898	8.089145	0.211239	31.5231	3.879444	$Y = 9.129C_0 + 2.768C_2 + 6.450C_4 + 46.062$

Table 7. Number of predictions outside 15% or 20% in absolute values (MAE%) of the real $AUC_{0\text{-}24}$ obtained with C0 and the selected LSS (C0-1-4).

N°	Formulation/Transplant type		20% C0	20% LSS	15% C0	15% LSS
20	Prograf®	Liver	3	0*	6	1*
21		Kidney	2	0	9	0*
20	Advagraf®	Liver	4	1	6	4
21		Kidney	5	0*	7	1*
41	Liver		6	1*	11	5*
41	Kidney		8	0*	17	1*
41	Prograf®		8	0*	16	2*
41	Advagraf®		9	2*	15	2*
82	Global		17	2*	31	8*

*$P < 0.05$ for the comparison LSS using time extraction points at 0, 1, and 4 h vs. C0.

Table 8. Number of predictions outside 15% or 20% in absolute values (MAE%) of the real $AUC_{0\text{-}24}$ obtained with C0 and LSS (C0-1-3) for all possible cases.

N°	Formulation/Transplant type		20% C0	20% LSS	15% C0	15% LSS
20	Prograf®	Liver	3	1	6	2*
21		Kidney	2	0	9	0*
20	Advagraf®	Liver	4	0*	6	0*
21		Kidney	5	1*	7	2*
41	Liver		6	0*	11	4*
41	Kidney		8	0*	17	7*
41	Prograf®		8	0*	16	3*
41	Advagraf®		9	3*	15	5*
82	Global		17	3*	31	13*

*$P < 0.05$ for the comparison LSS using time extraction points at 0, 1, and 3 h vs. C0.

The choice between the selected LSSs should be based on the type of patient and should take into account practical considerations. The use of different LSSs for each formulation and each transplant type (2 or 4 different LSSs) provides a better predictive performance, but is more complex and can be confusing and prone to errors in clinical practice. To select a unique LSS for the use with any type of formulation or transplant is simpler, but its performance is slightly poorer, particularly in the case of liver transplants. In our opinion, the use of a global LSS would be preferred for the use in routine clinical practice because the differences between the selected LSSs are low, and a global LSS would reduce possible mistakes in the selection of an appropriate LSS for each formulation and transplant type. A global LSS is also more convenient from a practical point of view. However, the selection of a specific LSS can be driven by other considerations; for example, the LSS C0-1-3 can be managed more easily than, for example, C0-1-4, a scheme that has also been proposed in adults by different authors [25], thus allowing the use of the same LSS in both populations.

In conclusion, we have developed the first LSS to predict the $AUC_{0\text{-}24}$ of tacrolimus in children and adolescents with a kidney or liver transplant after treatment with Prograf® and/or Advagraf®. This could be useful for both routine monitoring and for research purposes.

Authorship

GNA-P: designed research/study, collected data, analyzed data, and wrote the paper. RL: designed research/study, analyzed data, and wrote the paper. NLA-S: collected data, analyzed data, and wrote the paper. LE-R, CFC, CD and GMB: collected data. AJC-S: designed research/study and wrote the paper.

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References

1. Mendonza AE, Zahir H, Gohh RY, Akhlaghi F. Tacrolimus in diabetic kidney transplant recipients: pharmacokinetics and application of a limited sampling strategy. *Ther Drug Monit* 2007; **29**: 391.
2. van der Meer AF, Marcus MA, Touw DJ, Proost JH, Neef C. Optimal sampling strategy development methodology using maximum a posteriori Bayesian estimation. *Ther Drug Monit* 2011; **33**: 133.
3. Wallemacq P, Armstrong VW, Brunet M, *et al.* Opportunities to optimize tacrolimus therapy in solid organ transplantation: report of the European consensus conference. *Ther Drug Monit* 2009; **31**: 139.
4. Hon YY, Chamberlain CE, Kleiner DE, *et al.* Evaluation of tacrolimus abbreviated area-under-the-curve monitoring in renal transplant patients who are potentially at risk for adverse events. *Clin Transplant* 2010; **24**: 557.
5. Zhu L, Wang H, Rao W, Qu W, Sun L. A limited sampling strategy for tacrolimus in liver transplant patients. *Int J Clin Pharmacol Ther* 2013; **51**: 509.
6. Gaston RS, Hudson SL, Ward M, Jones P, Macon R. Late renal allograft loss: noncompliance masquerading as chronic rejection. *Transplant Proc* 1999; **31**: 21S.
7. EPAR 2007. *Scientific Discussion*. European Medicines Agency: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR__Scientific_Discussion/human/000712/WC500022237.pdf
8. Comuzzi C, Lorenzin D, Rossetto A, *et al.* Safety of conversion from twice-daily tacrolimus (Prograf) to once-daily prolonged-release tacrolimus (Advagraf) in stable liver transplant recipients. *Transplant Proc* 2010; **42**: 1320.
9. Sańko-Resmer J, Boillot O, Wolf P, Thorburn D. Renal function, efficacy and safety postconversion from twice- to once-daily tacrolimus in stable liver recipients: an open-label multicenter study. *Transpl Int* 2012; **25**: 283. Epub 2012 Jan 13.
10. Burra P, Germani G, Gnoato F, *et al.* Adherence in liver transplant recipients. *Liver Transpl* 2011; **17**: 760.
11. Carcas-Sansuán AJ, Hierro L, Almeida-Paulo GN, *et al.* Conversion from Prograf to Advagraf in adolescents with stable liver transplants: comparative pharmacokinetics and 1-year follow-up. *Liver Transpl* 2013; **19**: 1151.
12. Carcas-Sansuán AJ, Espinosa-Román L, Almeida-Paulo GN, *et al.* Conversion from Prograf to Advagraf in stable paediatric renal transplant patients and 1-year follow-up. *Pediatr Nephrol* 2014; **29**: 117.
13. Wallemacq PE, Furlan V, Möller A, *et al.* Pharmacokinetics of tacrolimus (FH506) in pediatric liver transplant recipients. *Eur J Drug Metab Pharmacokinet* 1998; **23**: 367.
14. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1986; **1**: 307.
15. Montini G, Ujka F, Varagnolo C, *et al.* The pharmacokinetics and immunosuppressive response of tacrolimus in paediatric renal transplant recipients. *Pediatr Nephrol* 2006; **21**: 719.
16. Ihara H, Shinkuma D, Ichikawa Y, Nojima M, Nagano S, Ikoma F. Intra- and interindividual variation in the pharmacokinetics of tacrolimus (FK506) in kidney transplant recipients–importance of trough level as a practical indicator. *Int J Urol* 1995; **2**: 151.
17. Jørgensen K, Povlsen J, Madsen S, *et al.* C2 (2-h) levels are not superior to trough levels as estimates of the area under the curve in tacrolimus-treated renal-transplant patients. *Nephrol Dial Transplant* 2002; **17**: 1487.
18. Mahalati K, Belitsky P, Sketris I. Neoral monitoring by simplified sparse sampling area under the concentration-time curve: its relationship to acute rejection and cyclosporine nephrotoxicity early after kidney transplantation. *Transplantation* 1999; **68**: 55.
19. Delaloye JR, Kassir N, Lapeyraque AL, *et al.* Limited sampling strategies for monitoring tacrolimus in pediatric liver transplant recipients. *Ther Drug Monit* 2011; **33**: 380.
20. Zhao W, Fakhoury M, Baudouin V, Maisin A, Deschênes G, Jacqz-Aigrain E. Limited sampling strategy for estimating individual exposure of tacrolimus in pediatric kidney transplant patients. *Ther Drug Monit* 2011; **33**: 681.
21. Velickovic-Radovanovic RM, Paunovic G, Mikov M, *et al.* Clinical pharmacokinetics of tacrolimus after the first oral administration in renal transplant recipients on triple immunosuppressive therapy. *Basic Clin Pharmacol Toxicol* 2010; **106**: 505.
22. Barraclough KA, Isbel NM, Kirkpatrick CM, *et al.* Evaluation of limited sampling methods for estimation of tacrolimus exposure in adult kidney transplant recipients. *Br J Clin Pharmacol* 2011; **71**: 207.
23. Bouamara R, Shukerb N, Hesselinkb DA, *et al.* Tacrolimus predose concentrations do not predict the risk of acute rejection after renal transplantation: a pooled analysis from three randomized-controlled clinical trials. *Am J Transplant* 2013; **13**: 1093.
24. Saint-Marcoux F, Debord J, Undre N, Rousseau A, Marquet P. Pharmacokinetic modeling and development of Bayesian estimators in kidney transplant patients receiving the tacrolimus once-daily formulation. *Ther Drug Monit* 2010; **32**: 129.
25. Woillard JB, de Winter BC, Kamar N, Marquet P, Rostaing L, Rousseau A. Population pharmacokinetic model and Bayesian estimator for two Tacrolimus formulations–twice daily Prograf and once daily Advagraf. *Br J Clin Pharmacol* 2011; **71**: 391.
26. Benkali K, Rostaing L, Premaud A, *et al.* Population pharmacokinetics and Bayesian estimation of tacrolimus exposure in renal transplant recipients on a new once-daily formulation. *Clin Pharmacokinet* 2010; **49**: 683.
27. Niioka T, Miura M, Kagaya H, *et al.* A limited sampling strategy to estimate the area under the concentration-time curve of tacrolimus modified-release once-daily preparation in renal transplant recipients. *Ther Drug Monit* 2013; **35**: 228.