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ORIGINAL ARTICLE

Population pharmacokinetics of subcutaneous alemtuzumab in kidney transplantation

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Aim: Alemtuzumab is a monoclonal antibody used as induction immunosuppressive therapy in kidney transplantation. It targets CD52 on lymphocytes, inducing profound immune cell depletion upon administration. Owing to its off-label status in kidney transplantation, its pharmacokinetic characteristics are largely unknown in this setting, and its current fixed dosing algorithm originates from other populations. We developed a population pharmacokinetic model for alemtuzumab in kidney transplant recipients and investigated the potential of personalized alemtuzumab therapy.

Methods: In total, 362 pharmacokinetic observations drawn 0-165 days after transplantation were available from 61 adult kidney transplant recipients who received two consecutive doses of 15 mg alemtuzumab subcutaneously. A population pharmacokinetic model was developed using nonlinear mixed-effects modelling and applied to simulate various dosing regimens.

Results: The alemtuzumab concentration-time data were best described by a two-compartmental model with first-order absorption and parallel first-order and time-varying concentration-dependent elimination, with between-subject variability on the first-order elimination (39.6%) and central distribution volume (39.6%). Alemtuzumab pharmacokinetics varied with body size, rendering lighter individuals exposed to lympholytic alemtuzumab concentrations (>0.1 mg/L) for prolonged durations as compared to their heavier peers. This between-subject variability could be reduced through lean bodyweight-adjusted dosing, showing a twofold to threefold reduction in the slope of the median alemtuzumab exposure over the bodyweight range.

Conclusion: Alemtuzumab displays substantial pharmacokinetic variability in kidney transplant recipients, which may warrant a personalized treatment strategy. Lean bodyweight-adjusted dosing poses an option for individualized dosing, but further evaluation of its potential clinical benefit is warranted.

The authors confirm that the Principal Investigator for this paper is D.J.A.R. Moes.

KEYWORDS
alemtuzumab, kidney transplantation, personalized dosing, pharmacokinetics, population pharmacokinetic modelling

1 | INTRODUCTION

Alemtuzumab is a humanized monoclonal antibody (mAb) applied as induction immunosuppressive therapy in kidney transplantation.[1] It targets CD52, a membrane glycoprotein expressed on nearly all B and T cells, macrophages, natural killer cells and granulocytes.[1] Binding of alemtuzumab to CD52 triggers complement-dependent cytotoxicity, antibody-dependent cytotoxicity and apoptosis, yielding a rapid and profound lymphocyte depletion upon administration which lasts several months up to years.[2–7]

Alemtuzumab is registered for relapse-remitting multiple sclerosis (MS), but is also available for adult solid organ transplant recipients via an off-label use procedure. In kidney transplant recipients, alemtuzumab induction therapy has shown efficacy similar to basiliximab and rabbit-derived antithymocyte globulin in terms of graft and patient survival, with slightly favourable acute rejection outcomes but moderately higher risk of infection.[1] Alemtuzumab is typically prescribed as a fixed dose of 30 mg administered intravenously or subcutaneously just before transplantation and, if divided over two doses of 15 mg, approximately 24 h thereafter.[1] Notably, in kidney transplantation, no formal dose-finding studies have been conducted, no exposure-effect relationship has been established and only limited information on its pharmacokinetic (PK) characteristics in this setting is available.[1,8] Consequently, the current dosing strategy is based mainly on experience from other patient populations, including MS, haematopoietic stem cell transplantation (HSCT) and chronic lymphocytic leukaemia (CLL). In these populations, considerable PK variability is apparent, with between-subject variabilities in alemtuzumab clearance, distribution and absorption of 36-104%,[9–13] 26-84%[9–12,14] and 73-101%,[11,12] respectively.

Furthermore, only limited evidence on exposure-response relationships of alemtuzumab is available. In vitro experiments have indicated lympholytic capabilities at alemtuzumab concentrations >0.1 mg/L.[15] Riechmann et al[15] incubated ^{51}Cr-labelled, activated peripheral blood mononuclear cells with a range of alemtuzumab concentrations and determined at which concentration ^{51}Cr release (i.e., cell death) occurred. These findings were supported by Marsh et al,[16] who suggested a lympholytic threshold of 0.10-0.16 mg/L in vivo, demonstrating threefold higher graft *vs* host disease incidence in HSCT recipients with alemtuzumab concentrations ≤0.15 *vs* >0.16 mg/L at the time of graft infusion. Moreover, these authors reported delayed lymphocyte recovery for patients displaying peritransplant alemtuzumab concentrations >0.57 mg/L and suggested a target range of 0.2-0.4 mg/L.[16] These cut-off values were determined at the time of graft infusion, 10-22 days after alemtuzumab administration, given either as a weight-adjusted or escalated dose on five consecutive days.[16] In another study in HSCT recipients, Loeff et al[17] showed that CD52-positive T cells remain absent at alemtuzumab concentrations >0.7 mg/L. Hence, a therapeutic range of 0.15-0.6 mg/L has been proposed for HSCT recipients,[12] demonstrating increasingly clear relations of alemtuzumab exposure with lymphocyte recovery and clinical outcomes in this setting. These findings have supported a personalized approach, with body size[9–12] and baseline lymphocyte count[9,11] as potential markers to guide individualized alemtuzumab therapy in HSCT, and provided a rationale for exploring a model-informed therapeutic drug monitoring (TDM)-guided dosing strategy.[18] Similarly, an exposure-response relationship has been suggested for alemtuzumab in CLL,[14] in which the probability of treatment response was dependent on alemtuzumab exposure.

What is already known about this subject

- Alemtuzumab is a humanized monoclonal antibody used as induction immunosuppressive therapy in kidney transplant recipients by means of an off-label use procedure.
- Alemtuzumab targets the CD52 cell surface membrane protein on immune cells, inducing rapid and profound immune cell depletion upon administration which lasts several months to years.
- Concerns about alemtuzumab's current fixed dosing strategy in kidney transplantation have been raised, as no formal dose-finding studies have been performed in this setting and substantial between-subject variability in lymphocyte recovery is observed after alemtuzumab induction therapy in kidney transplant recipients.

What this study adds

- We developed a population pharmacokinetic model for alemtuzumab in kidney transplant recipients, demonstrating considerable interindividual variability in alemtuzumab pharmacokinetics in this population.
- Alemtuzumab's pharmacokinetic variability is partly explained by variability in body size and predisposes lightweight individuals to higher and prolonged alemtuzumab exposure as compared to their heavier peers.
- Body size-adjusted dosing can be applied to personalize alemtuzumab therapy in the kidney transplantation setting, but further substantiation of the potential clinical benefit of this dosing strategy is warranted.

Unfortunately, translation of these pharmacometric studies to the kidney transplant setting is complicated by divergent dosing algorithms, concomitant immunosuppressive therapy, patient characteristics and CD52 expression. These HSCT patients received sequential body size-adjusted cumulative dosages of 0.2-1.5 mg/kg[10,12,13] or 45 mg/m^2 [11] administered over 4-5 consecutive days 7-15 days before graft infusion.[10–13] By contrast, MS patients received fixed dosages of 12-24 mg/day on five consecutive days, followed by 12-24 mg/day on three consecutive days 12 months thereafter.[9] For CLL, patients received dosages of 0.3-50 mg thrice-weekly over a course of 6-12 weeks, administered as multiple fixed dosages or by dose escalation.[14] Furthermore, in tandem with alemtuzumab induction, kidney transplant recipients receive concomitant oral immunosuppressive maintenance therapy, typically comprising a calcineurin inhibitor, antimetabolite and prednisolone. Similarly, HSCT recipients in these studies received concomitant immunomodulating therapy with sirolimus, cyclosporine or mycophenolate, combined with melphalan, fludarabine, cyclophosphamide or methotrexate.[10–13] The additional immunomodulating effects of these agents may impact CD52 expression and thereby affect the degree of CD52 expression-dependent target-mediated drug disposition (TMDD) of alemtuzumab.[14] By contrast, these MS and CLL patients received no concomitant immunomodulating therapy[9,14] and will thus likely display different extents of TMDD than seen in HSCT or kidney transplantation. Additionally, whereas HSCT, MS and kidney transplant patients typically show baseline absolute lymphocyte counts within the normal range,[9–13] and thus similar CD52 expression, baseline absolute lymphocyte counts can be up to tenfold higher in CLL patients.[14] This will also affect the degree of TMDD in these patients. Finally, differences between paediatric and adult patients and intravenous and subcutaneous administration likely add to the complexity of comparing alemtuzumab PK from these studies with the adult kidney transplantation setting.

Taken together, these population divergences, increasingly personalized alemtuzumab dosing strategies in other populations and clinical observations of substantial between-subject variability in immune cell recovery after alemtuzumab therapy in kidney transplantation[2,6,7] call for a thorough evaluation of the current fixed dosing strategy for alemtuzumab in kidney transplantation. Here, we describe, for the first time, the population PK of subcutaneous alemtuzumab in adult kidney transplant recipients. The between-subject PK variability of alemtuzumab is characterized and clinical patient characteristics to explain this variability are evaluated, which may serve as markers to guide personalized alemtuzumab therapy.

2 | METHODS

2.1 | Patients

All data originated from adult kidney transplant recipients who participated in the Triton study (NCT02057965).[7,19] In short, the Triton study was a randomized clinical trial, powered to include 70 de novo adult kidney transplant recipients. Participants received either autologous mesenchymal stromal cell (MSC) therapy with early tacrolimus withdrawal or continuous tacrolimus therapy without MSCs (control). Both groups received low-dose prednisolone, trough concentration (C_0)-guided everolimus therapy targeted at 6-8 μg/L and C_0-guided tacrolimus therapy targeted at 10-12 μg/L up to 6 weeks after transplantation. In the MSC group, participants received 1.5×10^6 autologous bone marrow MSCs per kilogram of total bodyweight intravenously at weeks 6 and 7 after transplantation, with tacrolimus therapy halved and then withdrawn in weeks 6 and 7, respectively. In the control group, C_0-guided tacrolimus therapy was continued beyond 6 weeks after transplantation, targeted at 6-8 μg/L for the remainder of the study. Induction immunosuppressive therapy for all participants consisted of two subcutaneous bolus injections containing 15 mg of alemtuzumab (Campath, Sanofi Genzyme, MA, USA) each, administered just before transplantation and 24 h thereafter. The study was approved by the Medical Ethical Committee of Leiden University Medical Center and all patients gave written informed consent.

2.2 | Samples and bioanalytics

Blood samples were collected 1 day before transplantation (baseline) and at 4, 6, 8 and 12 weeks thereafter. For a selection of patients, additional leftover samples from routine clinical chemistry assessments and/or immunosuppressant TDM could be obtained during the first days up to 26 weeks after transplantation.

Alemtuzumab was quantified in serum using a customized enzyme-linked immunosorbent-based assay (ELISA) based on human anti-alemtuzumab antibodies (NC Geoff Hale Developments, Oxford, UK).[20] The assay was developed and validated in accordance with the European Medicines Agency guideline on bioanalytical method validation[21] using seven calibration standards ranging between 0.01 and 0.039 mg/L, and two quality controls of 0.03 and 0.12 mg/L. The provisional lower and upper quantification limits of the assay for patient samples were set at 0.01 and 0.025 mg/L, respectively. Before analysis, quality controls and patient samples exceeding the highest calibration standard were diluted to concentrations within the quantification limits of the assay. The within-run and between-run accuracy and precision (expressed as coefficient of variation; CV%) were 96-105% and 3-9CV%, and 86-95% and 5-14CV%, respectively.

Baseline absolute cell counts were obtained by flow cytometry analysis using the BD Multitest kit (BD Biosciences, Franklin Lakes, NJ, USA).

2.3 | Population pharmacokinetic modelling

2.3.1 | Base model

A nonlinear mixed-effects model was developed to describe the population PK of alemtuzumab. For the base model, one- and

two-compartmental structures were evaluated to find the best fit for the concentration-time data. Standard and time-lagged zero- and first-order absorption, transit compartment absorption, and parallel and sequential absorption models were explored to describe alemtuzumab absorption,[22] and zero- and first-order elimination were evaluated to capture its elimination. Additionally, alemtuzumab elimination via TMDD was considered. Typically, the Michaelis-Menten model (Equation 1) is used to describe the TMDD of mAbs.[23,24] In case of time-varying antigenic mass, however, this model requires (a proxy for) the antigenic mass over time to capture the time-varying TMDD.[25] Because of the swift CD52 depletion after alemtuzumab administration, a decrease of the maximal elimination rate (V_{max}) over the first days was considered very likely. We thus evaluated a Michaelis-Menten model in which the time-varying elimination was incorporated using an exponential decay function (Equation 2), as described previously.[26] In these equations, MM is the Michaelis-Menten elimination, MM_t the time-varying Michaelis-Menten elimination, V_{max} is the maximal elimination rate, C is the concentration in the central compartment, K_m is the concentration at which the elimination rate is 50% of V_{max}, K_{dec} is a decay constant and t is time.

$$MM = \frac{V_{max} \times C}{K_m + C}, \quad (1)$$

$$MM_t = \frac{V_{max} \times e^{K_{dec} \times t} \times C}{K_m + C}. \quad (2)$$

The absolute bioavailability of alemtuzumab was fixed to 100%, as no intravenous PK data were available. Any flow and volume parameter estimates should thus be interpreted as apparent parameter estimates. Between-subject and between-occasion PK variability were modelled in an exponential manner, and additive, proportional and combined error models were evaluated to describe the residual unexplained variability.

2.3.2 | Covariate model

A covariate analysis was conducted to explain between-subject variability in alemtuzumab PKs and explore the potential of clinical markers to guide personalized alemtuzumab dosing.

Arguing from allometric theory and prior population PK studies in other populations receiving alemtuzumab or mAbs in general, body size was considered likely to affect alemtuzumab PK.[24] Hence, we evaluated a covariate model with allometric scaling to body size in which all flow parameters were exponentiated to 0.75 and linear proportionality was assumed for volume parameters. Total bodyweight (TBW), lean bodyweight (LBW) and blood volume at baseline were evaluated to find the optimal body size marker. Herein, LBW and blood volume were estimated from sex, TBW and height,[27,28] and body size was normalized to a male of 1.80 m and 70 kg. Additionally, previous population PK studies have demonstrated pretransplant lymphocyte counts to affect alemtuzumab PK,[9,11,14] particularly its TMDD. Baseline lymphocyte counts were thus evaluated as a covariate on V_{max}. Evidence on additional elimination pathways of alemtuzumab or mAbs in general is limited. Proteinuria, as a proxy for renal protein leakage, was considered a potential factor to affect alemtuzumab PK in kidney transplant recipients as renal damage is commonly observed in this population. Also, as lymphatic flow plays an important role in subcutaneous mAb absorption and its flow rate varies with age,[24] age was considered a potential factor to affect alemtuzumab absorption. Additionally, serum albumin was evaluated as a marker for neonatal F_c receptor recycling.[24] Finally, we evaluated the potential influence of treatment allocation in our study. Albeit any PK effect of a treatment divergence starting 6 weeks after alemtuzumab administration was considered unlikely, it was important to confirm this.

2.3.3 | Model selection and evaluation

Model selection was guided primarily by biological plausibility, parsimony and the objective function value change (ΔOFV) between a candidate model and its precursor, provided adequate model convergence, stability, visual diagnostics, parameter precision and shrinkage. A ΔOFV below −6.64 (df = 1, $P < .01$, assuming chi-squared distribution) was considered significant for model selection.

For the covariate analysis, initial exploration of covariate relationships was conducted using standard covariate plots, after which potentially influential covariates were further evaluated using stepwise covariate modelling. For the latter analysis, the ΔOFV thresholds for forward and backward selection were set at −3.84 ($P < .05$) and −6.64 ($P < .01$), respectively.

Visual model evaluation was conducted using standard goodness-of-fit plots, visual predictive checks (VPCs; N = 1000) and normalized prediction distribution errors (NPDE; N = 1000), and the robustness of the final parameter estimates was evaluated using a bootstrap procedure (N = 1000).[29]

2.3.4 | Simulations

The final model was used to perform simulations to characterize the extent and duration of alemtuzumab exposure in kidney transplant recipients and explore options to reduce its between-subject variability through individualized dosing.

First, the final model with maximum a posteriori Bayesian forecasting was applied to predict individual alemtuzumab PK curves. This enabled characterization of the basic individual PK statistics of alemtuzumab for our population, including the area under the concentration-time curve (AUC), elimination half-life ($T_{1/2}$), maximal concentration (C_{max}) and the time to C_{max} (T_{max}).

Second, although no therapeutic target range has been defined for alemtuzumab in kidney transplantation, it has been suggested that concentrations >0.1 mg/L are required for alemtuzumab to exert its lympholytic effect.[15] Additionally, peritransplant alemtuzumab concentrations >0.6 mg/L have been related to delayed lymphocyte

recovery in HSCT recipients.[16] It is likely that prolonged alemtuzumab exposure >0.6 mg/L will thus also be undesirable in the kidney transplantation setting. Hence, we used the individual predicted PK curves to calculate the mean number of days that patients displayed alemtuzumab concentrations above these thresholds of 0.1 and 0.6 mg/L.

Third, the efficacy and safety of alemtuzumab induction therapy on the individual patient level are likely related to the degree of lymphocyte depletion and subsequent duration until lymphocyte recovery. Thus, it is important to achieve a duration of lymphocyte depletion with an optimal balance of rejection prophylaxis and risk of infection for every individual patient. We hypothesized that the duration of lymphocyte depletion and concurrent time until lymphocyte recovery are partly dictated by alemtuzumab exposure, with between-subject variability in alemtuzumab exposure likely resulting in between-subject variability in lymphocyte dynamics and, ultimately, clinical outcomes. In that case, it can be of clinical interest to minimize the between-subject variability in alemtuzumab exposure across the kidney transplant recipient population. Additionally, various dosing algorithms have been applied for alemtuzumab induction in kidney transplantation, including cumulative fixed dosages of 20,[30] 30[1] and 60 mg,[1] administered either as one dosage of 100% just before transplantation or as two consecutive dosages of 50%, administered just before transplantation and 24 h thereafter. As no formal dose-finding studies have been performed for alemtuzumab in kidney transplantation, the optimal alemtuzumab dosage and dosing algorithm remain unknown. It is informative, however, to evaluate how alemtuzumab exposure varies across these different dosages and dosing algorithms. We thus investigated how the between-subject variability in alemtuzumab exposure relates to the current fixed cumulative dose of 30 mg and alternative cumulative dosages of 10-60 mg, explored alternative dosing algorithms to reduce the between-subject variability in alemtuzumab exposure and evaluated the PK differences between a single alemtuzumab dose of 30 mg *vs* two consecutive dosages of 15 mg, 24 h apart.

For each dosing scenario, the median alemtuzumab concentration and its 90% prediction interval were generated using Monte Carlo simulations (N = 500).

2.4 | Software and settings

Data handling, statistics and graphical visualization were performed in R 3.6.2 (R Project for Statistical Computing, Vienna, Austria) and RStudio 1.2.5019 (RStudio Inc., Boston, MA, USA). Nonlinear mixed-effects modelling was performed in NONMEM 7.4.4 (Icon Development Solutions, Ellicott City, MD, USA) using Perl-speaks-NONMEM (PsN) Toolkit 5.0.0 and Piraña 2.9.8 as the modelling interface.[31,32] The first-order conditional estimation method with η-ε interaction was applied for initial parameter estimation, using the differential equation solver ADVAN6 with $DES for models describing nonlinear PK behaviour. Dependent on the extent and informativeness of concentration-time data below the limit of quantification of the ELISA (BLQ), BLQ data were either omitted or retained and handled as per the M3 method using Laplacian conditional estimation with η-ε interaction.[33–35]

2.5 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY, and are permanently archived in the Concise guide to PHARMACOLOGY 2019/20.[36]

3 | RESULTS

3.1 | Patients and samples

Blood samples drawn between 0 and 165 days after transplantation were available from 61 kidney transplant recipients. Their clinical characteristics are summarized in Table 1. Five patients displayed one to four divergent alemtuzumab concentrations in their PK curve, with two showing increasing alemtuzumab concentrations during the elimination phase, one displaying a flat peak and two showing divergent BLQ data. These observations (N = 11, 3.0%) were considered physiologically unlikely and disproportionally influenced the parameter

TABLE 1 Baseline patient characteristics (N = 61)

Characteristic	N	Mean	Range
Sex			
Male	50 (82.0%)		
Female	11 (18.0%)		
Age (years)		49.9	19-74
Body size			
Blood volume (L)		6.07	4.16-7.86
Height (cm)		177	158-198
Lean bodyweight (kg)		60.3	40.2-79.2
Total bodyweight (kg)		81.6	54.6-119
Blood chemistry			
Albumin (g/L)		43.8	32-52
Urine chemistry			
Total protein (g/24 h)		1.87	0-11.6
Absolute cell count[a]			
Leukocytes (L^{-1}, $\times 10^9$)		7.85	3.74-15.1
Lymphocytes (L^{-1}, $\times 10^9$)		1.52	0.56-2.82
Treatment allocation			
Mesenchymal stromal cells	33 (54.1%)		
Control	28 (45.9%)		

[a]Absolute cell counts at baseline were available for 57/61 patients (93.4%).

estimation, and were thus excluded. In total, 362 PK observations were used for model development, of which 26 (7.2%) comprised BLQ observations (Figure 1).

3.2 | Pharmacokinetic modelling

3.2.1 | Base model

The parameter estimates of the base model are summarized in Table 2. A two-compartmental model with standard first-order absorption and parallel first-order and time-varying Michaelis-Menten elimination yielded the best fit of the concentration-time data. Between-subject variability was modelled on the central distribution volume and the first-order elimination, and a combined error model was used to describe the residual unexplained variability.

Likely owing to the scarcity of observations in the absorption phase, independent estimation of the volumes of the central and peripheral compartments resulted in unstable models in terms of parameter estimate uncertainty and convergence. However, disregarding the peripheral compartment altogether showed a clear model misspecification. Hence, the peripheral compartment was defined as a factor of the central compartment, as described previously.[10] Of note, this manner of parameterization assumes equal between-subject variability in the central and peripheral distribution volumes, which is physiologically unlikely. However, although discarded due to high relative standard errors (RSEs) and shrinkage, intermediate models with independently estimated central and peripheral distribution volumes did indicate a large degree of covariance (±75%) between the between-subject variabilities of both parameters, providing adequate justification for applying this manner of parameterization.

Additionally, some estimation difficulties were encountered regarding the Michaelis-Menten elimination parameters, showing RSE and shrinkage >30%. Fixing K_m resolved this and showed adequate visual diagnostics. Herein, several models incorporating fixed K_m values ranging around its initially estimated value were explored to find the K_m value associated with the lowest OFV. More complex or mechanistic approaches to capture alemtuzumab absorption and/or distribution were discarded, as these were unidentifiable, unstable or did not yield model improvement.

Modelling between-subject variability on the central distribution volume and the first-order elimination further improved the model. By contrast, between-subject variability was not identifiable for the bioavailability, absorption or Michaelis-Menten elimination parameters. Although several intermediate models with between-subject variability on V_{max} did yield lower OFV values in the range of −1880, these were associated with pronounced shrinkage and RSEs exceeding 50% and were thus discarded. No between-occasion variability in bioavailability or absorption was identifiable.

Estimation of the additive error resulted in RSEs >30%, likely because of the uncertainties associated with the BLQ data. The additive error was thus fixed relative to the lower limit of quantification (LOQ) of the assay, for which a value of 30% of the LOQ of the assay showed the lowest OFV as compared to alternative values between 0% and 100% of the LOQ.

3.2.2 | Covariate model

The parameter estimates of the final model are summarized in Table 2. A model including allometric scaling of the central distribution volume, first-order elimination, intercompartmental clearance and V_{max} to LBW showed the best fit of the concentration-time data. In this model, all flow parameters were exponentiated by 0.75, whereas linear proportionality was assumed for volume parameters.[37]

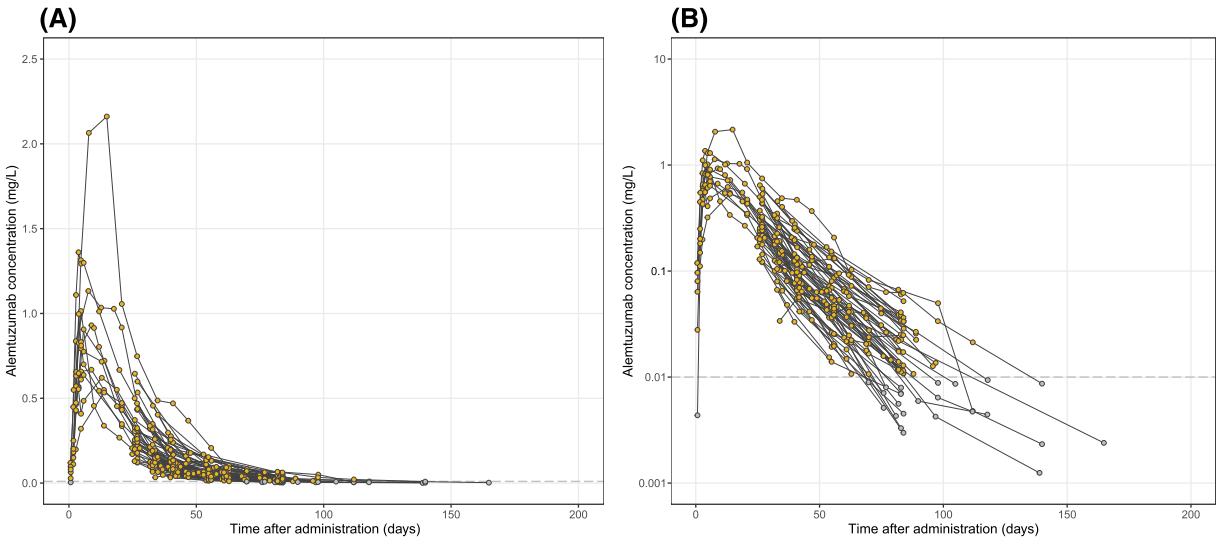

FIGURE 1 Alemtuzumab pharmacokinetics over time on (A) linear and (B) semilogarithmic scales. The dotted grey line indicates the lower quantification limit of 0.01 mg/L, with the observations below the lower quantification limit indicated in grey

TABLE 2 Population pharmacokinetic parameter estimates of the base and final models

	Base model			Final model			
Parameter[a]	Estimate	RSE (%)	Shrinkage (%)	Estimate	RSE (%)	Shrinkage (%)	Median estimate[b] [95% CI]
Population values							
F (%)	100	FIX		100	FIX		100 [FIX]
K_a (day^{-1})	0.0867	10		0.091	10		0.091 [0.074; 0.133]
V_c (L)	5.72	16		6.33[c]	16		6.33 [4.57; 10.5]
$CL_{first\text{-}order}$ (L/day)	1.43	6		1.41[c]	5		1.41 [1.25; 1.62]
V_{max} (mg/day)	10.5	26		10.6[c]	27		11.0 [6.22; 45.6]
K_m (mg/L)	0.391	FIX		0.395	FIX		0.395 [FIX]
K_{dec} (day^{-1})	−1.29	18		−1.32	14		−1.32 [−2.34; −0.95]
Q (L/day)	0.264	21		0.294[c]	21		0.292 [0.176; 0.485]
V_p (factor of V_c)	1.28	17		1.11	15		1.11 [0.72; 1.58]
Between-subject variability							
V_c (CV%)	39.6	30	21	33.0	25	25	33.0 [16.3; 52.2]
$CL_{first\text{-}order}$ (CV%)	39.6	10	1	35.5	9	1	35.4 [29.0; 41.3]
Random residual variability							
Proportional error (CV%)	20.5	5	17	20.4	5	17	20.2 [17.7; 22.2]
Additive error (mg/L)	0.003	FIX		0.003	FIX		0.003 [FIX]

Abbreviations: 95% CI, 95% confidence interval; $CL_{first\text{-}order}$, first-order elimination; CV%, coefficient of variation; F, bioavailability; FIX, fixed; K_a, absorption rate; K_{dec}, decay constant; K_m, concentration at which the elimination rate is at 50% of V_{max}; Q, intercompartmental clearance; RSE, relative standard error; V_c, central distribution volume; V_{max}, maximal elimination rate; V_p, peripheral distribution volume expressed as factor of the central distribution volume.

[a]As the bioavailability could not be estimated in the present study and was thus fixed to 100%, any flow or volume parameter estimates should be interpreted as apparent parameter estimates.

[b]Median parameter estimate and 95% confidence interval as derived from the bootstrap analysis (N = 1000).

[c]Allometrically scaled to a lean bodyweight of 57.4 kg, corresponding with a male with a total bodyweight of 70 kg and a height of 1.80 m, using allometric scaling exponents of 0.75 and 1 for flow and volume parameters, respectively.

The exploratory covariate plots indicated body size to affect alemtuzumab PK, with its first-order elimination and central distribution volume increasing with body size. These relationships were similar for TBW, LBW and blood volume, whereas height showed a less pronounced trend. Additionally, the first-order elimination appeared to increase with total urine protein. No relationships between the other covariates and alemtuzumab PK were discernible. Thus, TBW, LBW, blood volume and total urine protein were included for stepwise covariate modelling.

Inclusion of total urine protein on the first-order elimination did not reach statistical significance during the forward search and was thus excluded from further evaluation. By contrast, inclusion of the body size metrics on the first-order elimination and the central distribution volume did yield statistically significant improvement of the model in the forward and backward search. In the final covariate model, allometric scaling of the first-order elimination, central distribution volume, intercompartmental clearance and V_{max} to LBW resulted in a ΔOFV of −12.5 as compared to the base model, showing a very similar but slightly lower OFV change compared to a model with allometric scaling to TBW (ΔOFV −12.3). This coincided with improved visual diagnostics and moderate reductions of the between-subject variability in the central distribution volume (39.6% → 33.0%, −16.7%) and first-order elimination (39.6% → 35.5%, −10.4%) as compared to the base model.

A stepwise overview of the model development and the NONMEM code of the final model are available in the **Supporting Information**.

3.2.3 | Model evaluation

The visual diagnostics of the final model are depicted in Figures 2 and S1, and the bootstrap analysis is summarized in Table 2. The goodness-of-fit plots (Figure 2A-F) showed an adequate concordance of the observed and population and individual predicted alemtuzumab concentrations, and no particular trends of the conditional weighted residuals. Similarly, the VPC (Figure 2G,H) showed an acceptable overlay between the simulated and observed concentrations and the NPDE (Figure S1) showed no systematic bias, indicative of adequate description of the PK of alemtuzumab. Finally, the parameter estimates of the final model were all within 5% of the median parameter estimates of the bootstrap analysis, indicating good parameter estimate robustness.

Additionally, the derived parameter estimates for the first-order elimination, V_{max}, K_m and K_{dec} from the final model were used to

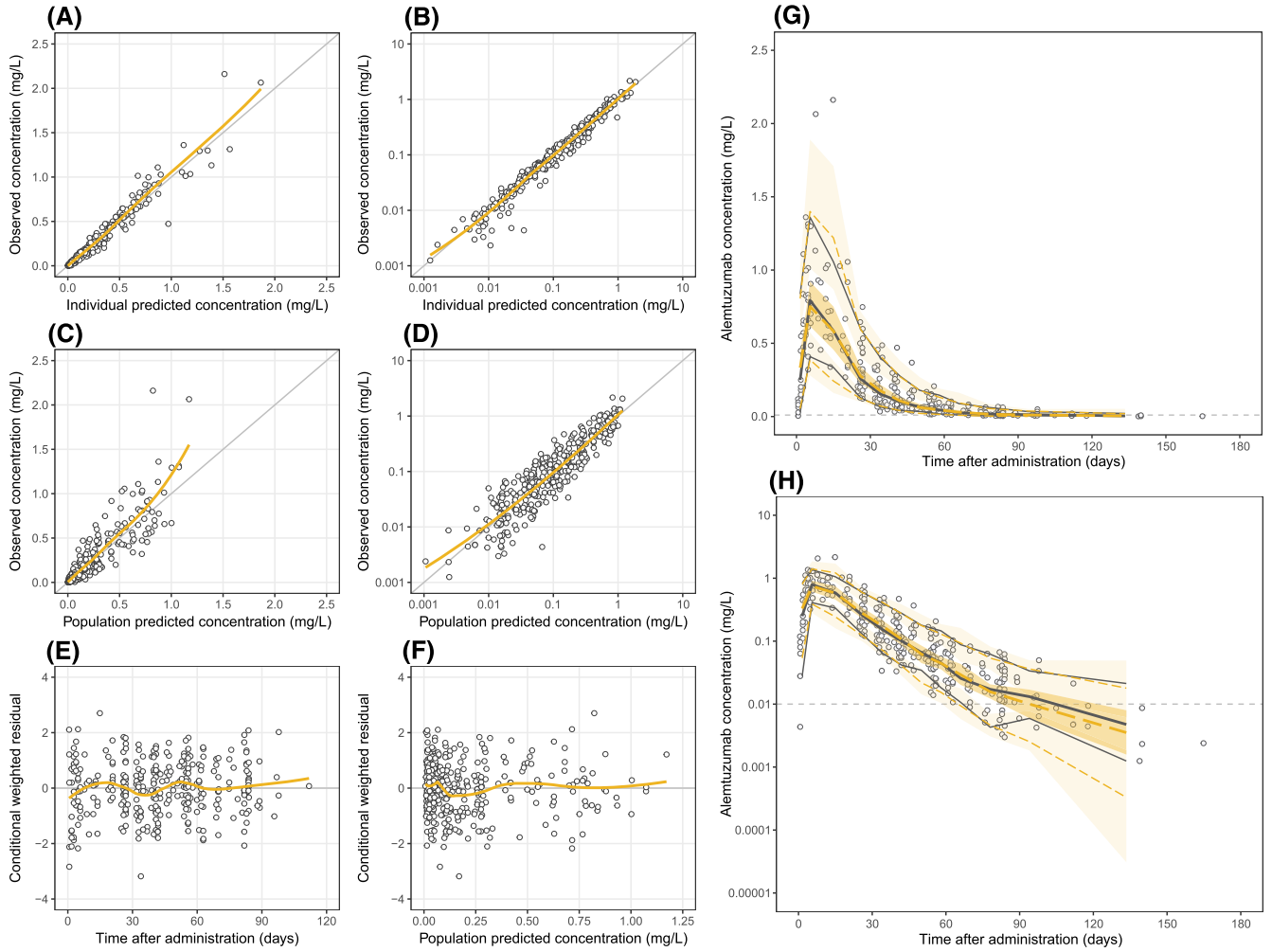

FIGURE 2 Visual diagnostics for the final model. (A, B) Observed *vs* individual predicted alemtuzumab concentrations on the linear and logarithmic scales. (C, D) Observed *vs* population predicted alemtuzumab concentrations on the linear and logarithmic scales. (E) Conditional weighted residuals over time. (F) Conditional weighted residuals over population predicted alemtuzumab concentrations. (G, H) Visual predictive check on the linear and semilogarithmic scales. In (A-F), solid gold lines represent the loess regression fit, whereas solid grey lines represent the lines of identity. In (G) and (H), the gold dotted lines and gold-shaded areas represent the 5th, 50th and 95th percentiles of the simulated data and their 95% confidence intervals, whereas the solid black lines represent the 5th, 50th and 95th percentiles of the observed data. The dotted grey line indicates the lower quantification limit

visualize the decrease of V_{max} over time (Figure 3A) and the contributions of the first-order and Michaelis-Menten elimination processes over the alemtuzumab concentration for the first days after administration (Figure 3B,C). Consistent with the swift lymphocyte depletion and concurrent reduction of the CD52 antigen mass after alemtuzumab induction, our results suggest that the contribution of the Michaelis-Menten elimination to the total alemtuzumab elimination is maximal directly after administration, with a rapid decrease to a negligible contribution beyond 4 days after administration.

3.2.4 | Simulations

First, the final model with maximum a posteriori Bayesian forecasting was used to predict the individual alemtuzumab curves from time zero up to 150 days after administration (Figure S2). These enabled derivation of the basic PK statistics of alemtuzumab in our population, displaying a mean $AUC_{0-\infty}$ of 22.1 ± 9.06 mg/L × days (range 8.82-53.5), $T_{1/2}$ of 2.97 ± 1.00 days (range 1.30-6.62), C_{max} of 0.88 ± 0.28 mg/L (range 0.44-1.87) and T_{max} of 7.38 ± 1.05 days (range 5.3-10.7).

Second, from the individual predicted PK curves, we calculated the mean number of days that patients displayed alemtuzumab concentrations above 0.1 and 0.6 mg/L. All patients reached lymphocytic concentrations (>0.1 mg/L), with an average duration of 42.7 ± 9.93 days (range 25.6-69.2 days). Concentrations exceeding the 0.6 mg/L threshold were reached in 54/61 (88.5%) patients, for an average duration of 13.0 ± 6.11 days (range 3.30-29.6 days). The time that patients displayed alemtuzumab concentrations above the 0.1 and 0.6 mg/L thresholds showed a clear correlation

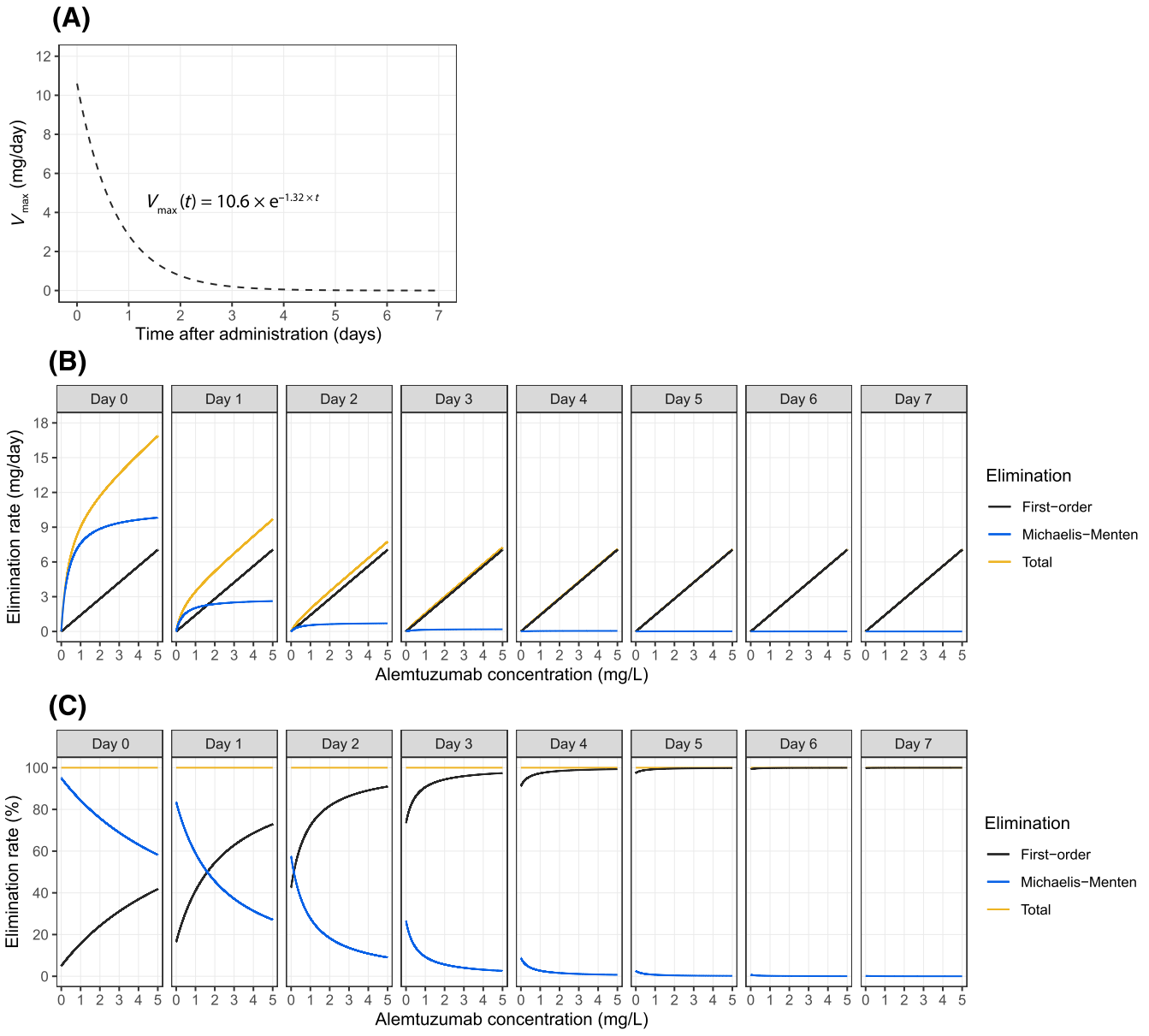

FIGURE 3 Visualization of the (A) maximal Michaelis-Menten elimination (V_{max}) and the (B) absolute and (C) relative contributions of the first-order and Michaelis-Menten elimination processes over the first 7 days after alemtuzumab administration, as calculated using Equation (2) with the estimated population values of $CL_{first\text{-}order}$, V_{max}, K_m and K_{dec}

with TBW (Figure 4A), with these times decreasing with increasing bodyweight. Furthermore, the times above the 0.1 and 0.6 mg/L thresholds were correlated with the individual predicted $AUC_{0\text{-}\infty}$ (Figure 4B), which thus harnesses informative value for prediction of the duration of lympholytic alemtuzumab exposure.

Third, we simulated the alemtuzumab PK associated with a single fixed dose of 30 mg and compared this profile with the current fixed dosing strategy comprising two consecutive doses of 15 mg, administered 24 h apart. For a male individual with a weight of 70 kg and a height of 1.80 m, both dosing strategies displayed highly similar PK profiles (Figure S3), with the single and split fixed dosing strategies resulting in median $AUC_{0\text{-}\infty}$ values of 20.4 ± 8.03 mg/L × days and 20.5 ± 8.17 mg/L × days, respectively. Thus, in terms of PK, these results suggest no particular disadvantages for using a single fixed dosing strategy as compared to the current split fixed dosing strategy.

Fourth and finally, we evaluated the alemtuzumab $AUC_{0\text{-}\infty}$ and C_{max} under various dosing regimens and explored options to minimize its between-subject variability. As expected, a clear positive relationship was apparent between the cumulative alemtuzumab dose and the $AUC_{0\text{-}\infty}$ and C_{max}, with the median $AUC_{0\text{-}\infty}$ and C_{max} for a male of 70 kg and a height of 1.80 m increasing from 6.73 ± 2.74 (range 1.80-18.3 mg/L × days) and 0.29 ± 0.08 mg/L (range 0.11-0.67 mg/L) for a cumulative fixed dose of 10 mg to 41.3 ± 17.0 (range 10.9-114 mg/L × days) and 1.78 ± 0.53 mg/L (range 0.68-4.21 mg/L)

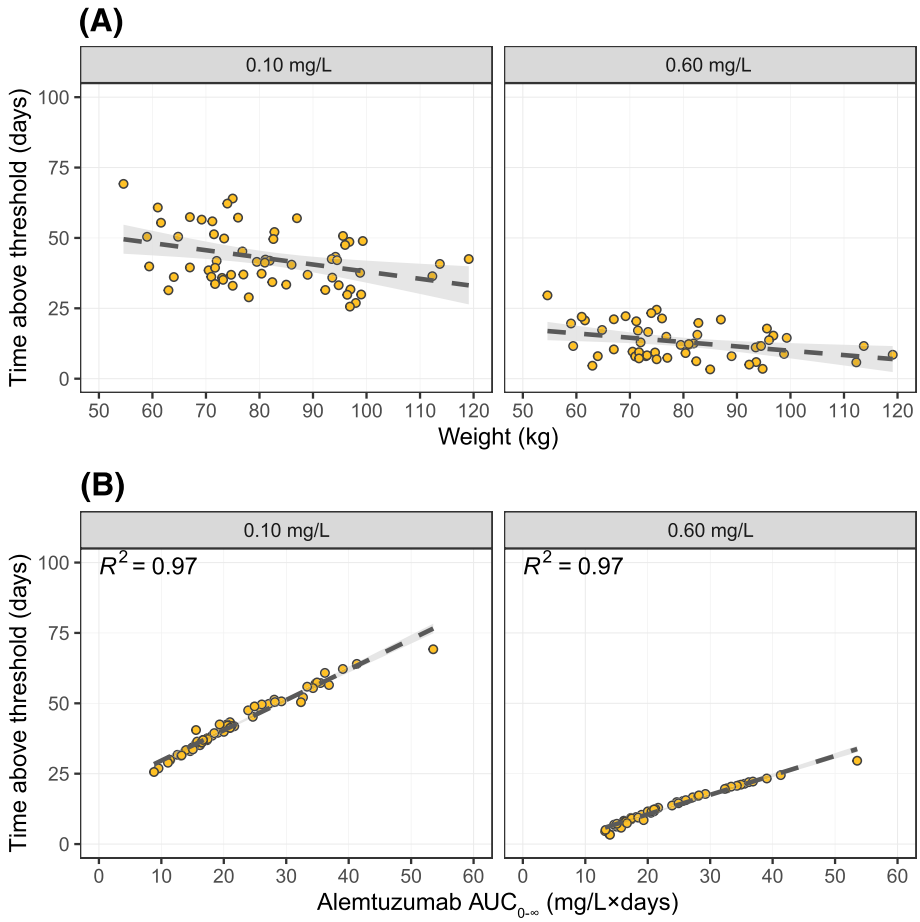

FIGURE 4 Correlation of (A) total bodyweight and the individual predicted number of days that patients displayed an alemtuzumab concentration exceeding 0.10 and 0.60 mg/L and (B) the individual predicted area under the alemtuzumab concentration-time curve from time zero to infinity ($AUC_{0-\infty}$) and the number of days that patients displayed an individual predicted alemtuzumab concentration exceeding 0.10 and 0.60 mg/L. Dashed black lines and grey-shaded areas depict the linear regression lines and their standard error

for 60 mg, respectively (Table S2). Furthermore, and most importantly, the observed inverse trend of the $AUC_{0-\infty}$ over the bodyweight range for the current fixed dosing strategy could be reduced with a LBW-adjusted dosing strategy (Figure 5). Although the LBW-adjusted dosing strategy displayed slight positive trends of the $AUC_{0-\infty}$ over the TBW range for the various dosages, the slopes of these trends were approximately twofold to threefold lower than for the fixed dosing strategy, indicating a substantial reduction in the body size-dependent between-subject variability in alemtuzumab exposure. This poses LBW-adjusted dosing as an alternative dosing strategy to equalize the alemtuzumab exposure across the kidney transplant recipient population.

Finally, to investigate the clinical utility of the C_{max} or $AUC_{0-\infty}$ as a potential marker for personalized alemtuzumab therapy, in case a C_{max} or $AUC_{0-\infty}$ target range is derived in the future, we evaluated the informativeness of alemtuzumab concentrations quantified at days 1-7 after alemtuzumab administration for their corresponding C_{max} or $AUC_{0-\infty}$. As depicted in Figure S4, the individual predicted alemtuzumab concentrations at day 6 or 7 after administration harness predictive value for their corresponding C_{max} and $AUC_{0-\infty}$, whereas those quantified before day 6 do not. This could allow for model-based approximation of the C_{max} or $AUC_{0-\infty}$ in the first week after transplantation, in which patients are still hospitalized, and perhaps administer an additional dose if the C_{max} or $AUC_{0-\infty}$ is below the desired target range.

All simulated alemtuzumab PK curves and corresponding $AUC_{0-\infty}$ and C_{max} values are provided in Figure S5 and Table S2, respectively.

4 | DISCUSSION

In this study, a population PK model for subcutaneously administered alemtuzumab in adult kidney transplant recipients was developed for the first time. Similar to what has been previously observed in other populations, alemtuzumab exhibits considerable PK variability in kidney transplant recipients, with between-subject variabilities in elimination and distribution of approximately 40%. Its PK variability is partly explained by body size, with alemtuzumab elimination and distribution increasing with body size. The concurrent inverse relation between body size and alemtuzumab exposure renders lightweight individuals at an extended exposure to lympholytic alemtuzumab concentrations as compared to their heavier peers with the current fixed dosing algorithm. We demonstrated that this variability can be equalized with a LBW-adjusted dosing algorithm, although the potential clinical benefit of this approach could not be substantiated.

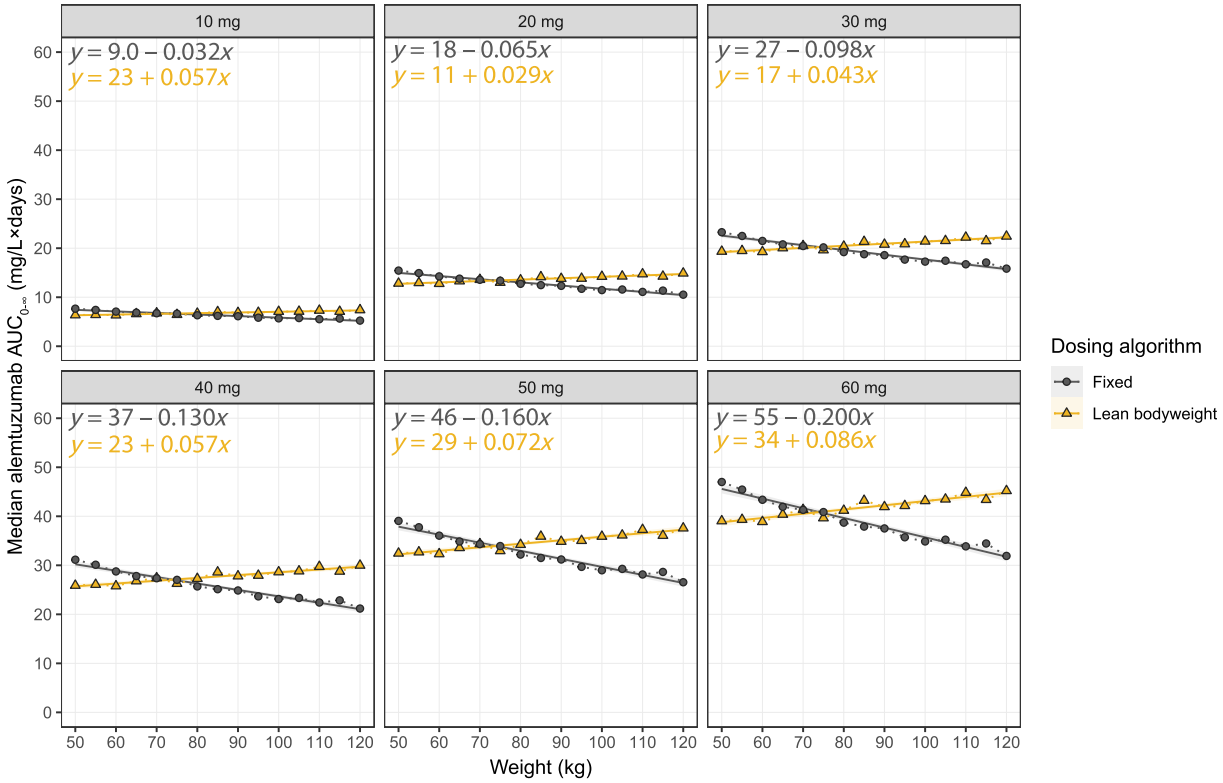

FIGURE 5 Median simulated $AUC_{0-\infty}$ over the bodyweight range for doses of 10, 20, 30, 40, 50 and 60 mg. The simulations for the fixed dosing strategy (administered as two subsequent dosages of 50% of the cumulative dose just before transplantation and 24 h thereafter) are shaded black, whereas those for the lean bodyweight (LBW)-adjusted dosing strategy, standardized to the LBW of a male of 70 kg and 1.80 m (administered as two subsequent dosages of 50% of the cumulative dose just before transplantation and 24 h thereafter), are shaded gold. Solid grey and gold lines and the grey- and gold-shaded areas around the solid lines depict the linear regression fits and their standard errors, with the concurrent linear regression fit equations included on the plot in corresponding colours

Alemtuzumab PK was best described by a two-compartmental model with first-order absorption and parallel first-order and time-varying Michaelis-Menten elimination. Alemtuzumab displayed an apparent first-order elimination of 1.41 L/day and an apparent steady-state distribution volume of 13.4 L. Although direct comparison of our parameter estimates to those of previous population PK studies is complicated by divergent dosing algorithms, CD52 expression, patient characteristics and concomitant immunosuppressive therapy, it is informative to see how alemtuzumab PK differ across populations. Our parameter estimates are in line with Dong et al,[12] who reported a clearance of 1.91 L/day/70 kg and distribution volume of 17.4 L/70 kg in paediatric and young adult HSCT recipients. Also, Li et al[9] showed a sample size-weighted mean clearance of 1.39 L/day and distribution volume of 14.1 L in adult MS patients. Similarly, Admiraal et al[10] reported a population clearance of 0.25 L/day and steady-state distribution volume of 3.62 L in paediatric HSCT recipients, for which weight standardization to an individual of 70 kg[38] yields a clearance estimate (0.71 L/day) that is lower than seen in kidney transplantation, but a comparable steady-state distribution volume (14.7 L). Similarly, Furstenau et al[13] observed a clearance of 0.62 L/day and distribution volume of 10.4 L in adult HSCT recipients. Although the latter estimates are more or less in the same range as ours, a bioavailability of 48-55%[1,11] should be considered when comparing intravenous and subcutaneous alemtuzumab PK. By contrast, Bhoopalan et al reported a clearance of 0.29 L/day and steady-state distribution volume of 3.87 L for subcutaneous alemtuzumab in paediatric and young adult HSCT recipients,[11] whereas Mould et al[14] reported a steady-state distribution volume of 52.8 L in adult CLL patients, which seems uncharacteristically large for a mAb.[23,39,40] We parameterized alemtuzumab absorption after subcutaneous administration as a first-order process, which is consistent with the model by Bhoopalan et al.[11] Alternatively, Dong et al[12] used a sequential process of zero-order and first-order absorption. Efforts to fit such an absorption model to our data, however, resulted in overparameterization. Interestingly, our K_a of 0.093 day^{-1} indicated slower absorption than the 0.78-1.90 day^{-1} reported by Bhoopalan et al and Dong et al,[11,12] and also seems low when compared to the 0.125-1.45 day^{-1} reported for other subcutaneously administered mAbs.[22] Our V_{max} of 10.6 mg/day and K_m of 0.395 mg/L were similar to the values of 24.5 mg/day and 0.338 mg/L reported by Mould et al in CLL,[14] with their higher V_{max} likely associated with the elevated CD52 antigen burden in CLL patients. Also, these authors parameterized their V_{max} to decrease with decreasing lymphocyte count, which is consistent with our time-dependent parameterization

of V_{max} using an exponential decay constant (K_{dec}). By contrast, Admiraal et al reported a K_m of 1.38 mg/L and V_{max} of 0.42 mg/day, independent of time or lymphocyte count, of which the latter also remains substantially lower than our estimate (1.20 mg/day) after weight standardization to an individual of 70 kg.[38] This may be related to their time- and lymphocyte-independent parameterization of V_{max}.

Whereas Li et al, Mould et al and Furstenau et al reported alemtuzumab PK to be dependent on baseline lymphocyte count,[9,13,14] we found no such effect. Perhaps this is explained by the rather narrow range of baseline absolute lymphocyte counts (0.56-2.82 $\times$ 10^9 L^{-1}) in our population, as compared to the ranges reported by Mould et al[14] (16.9-92.0 $\times$ 10^9 L^{-1}), Furstenau et al[13] (approximately 0-8 $\times$ 10^9 L^{-1}) and Li et al[9] (1.05 $\times$ 10^9 L^{-1} $\pm$ 0.34 SD to 2.01 $\times$ 10^9 L^{-1} $\pm$ 0.69 SD, translating to approximately 0.17-3.79 $\times$ 10^9 L^{-1} when calculated as a 99% interval from their reported mean values and standard deviations). Accordingly, our findings are in line with Admiraal et al, who also found no effect of baseline lymphocyte counts on alemtuzumab PK and reported a narrow baseline absolute lymphocyte count range of 0.53-1.5 $\times$ 10^9 L^{-1}.[10] By contrast, Dong et al[12] reported a slightly broader baseline absolute lymphocyte count range of 0.06-6.0 $\times$ 10^9 L^{-1} but did not find an effect of baseline lymphocyte counts on alemtuzumab PK. Similarly, our finding that alemtuzumab PK were not affected by proteinuria may be related to the limited number of patients displaying excessive proteinuria in our population. Although the extent of proteinuria in our population is consistent with the general kidney transplantation population,[41] we cannot rule out a potential effect of renal protein leakage on alemtuzumab PK in patients with excessive proteinuria. Finally, we found alemtuzumab PK to be dependent on body size, which is consistent with all but two of the other studies.[9–12]

Our study highlights body size-adjusted dosing as a potential alternative to the current fixed dosing algorithm for alemtuzumab in kidney transplant recipients. This is consistent with previous studies that reported on the potential of body size-adjusted dosing to reduce PK variability for mAbs that display a high proportionality of elimination and distribution to body size.[42,43] In particular, Wang et al demonstrated that body size-adjusted dosing results in reduced variability in exposure as compared to fixed dosing when monoclonal antibody clearance displays an exponential covariate relationship with body size with an exponent >0.5.[42] Although our limited sample size thwarted reliable estimation of this exponent in our population,[44] the model improvement associated with the inclusion of the covariate relationships with respective exponents of 0.75 and 1.00 did indicate high proportionality of alemtuzumab elimination and distribution to body size. We demonstrated that with the current dosing regimen, lightweight individuals undergo extended exposure to lympholytic alemtuzumab concentrations (>0.1 mg/L) and also display prolonged exposure to alemtuzumab concentrations associated with delayed lymphocyte recovery (>0.6 mg/L) as compared to their heavier peers. This may leave lighter individuals at an increased risk of overimmunosuppression, which is likely associated with a higher susceptibility to infection. Contrarily, heavier individuals may experience underimmunosuppression, although the consequences of slight underimmunosuppression at a cumulative dosage of 30 mg may be moderate, as a previous study reported similar allograft rejection rates for cumulative dosages of 20 *vs* 30 mg alemtuzumab in 46 adult kidney transplant recipients.[30] Our findings are also consistent with Willicombe et al, who retrospectively compared TBW-adjusted dosing (0.4 mg/kg) *vs* fixed dosing (30 mg) of alemtuzumab in 888 kidney transplant recipients.[45] These authors reported statistically significantly higher lymphocyte counts and fewer infections with body size-adjusted dosing during the first year after transplantation, with similar allograft and patient survival in both groups.[45] The clinical interpretation of our findings, however, is complicated by the absence of a clearly defined exposure-response relationship or therapeutic range for alemtuzumab in kidney transplantation. This renders it currently unclear if the divergence in exposure across the body size range is clinically relevant and whether a LBW-adjusted dosing approach would be of added clinical value. Whereas the report by Willicombe et al[45] suggested a clinical benefit of exposure equalization across the bodyweight range, body size accounted for only 16.7% and 10.4% of the variability in alemtuzumab elimination and distribution in our study, respectively. Thus, while a LBW-adjusted dosing algorithm may result in more equalized exposure on a population level, the effect on the individual patient level may be marginal considering the residual between-subject variability. Also, as body size-adjusted dosing typically comes at reduced cost effectiveness, medication safety and clinical practicality,[28] it is important to also evaluate whether its potential clinical benefit outweighs these disadvantages.

Our study has some limitations. First and foremost, the absence of data on lymphocyte dynamics and clinical outcomes thwarted the substantiation of the clinical implications of our findings. This renders the optimal extent and duration of alemtuzumab exposure, and thus the optimal dosing regimen, still unknown. However, our study is the first to thoroughly characterize alemtuzumab PK in this population, and thus provides an important basis for future studies on the rationale for personalized alemtuzumab therapy. Second, the scarcity of the PK data in the first 4 weeks after transplantation rendered parameterization of alemtuzumab absorption and distribution complicated, which could have been optimized in a PK-centred study. Contrarily, this was the most practical solution to capture alemtuzumab absorption and distribution in this non-PK-centred study. Third, while no sudden, dramatic drops in the individual alemtuzumab PK profiles were observed, no quantitative data on anti-alemtuzumab antibody formation were available. As neutralizing antidrug antibodies can detrimentally affect mAb elimination, this comprises an important entity to consider when characterizing mAb PK.[26] However, whereas neutralizing anti-alemtuzumab antibodies are not uncommon in MS,[46] the short treatment course and concomitant oral immunosuppressive therapy likely yield a lower incidence of neutralizing anti-alemtuzumab antibodies in solid organ transplantation.[1,47] Fourth, while we demonstrated that LBW-adjusted dosing can be applied

to reduce the between-subject variability in alemtuzumab PK across the bodyweight range, LBW-adjusted dosing did show a slight positive trend over the bodyweight range. Although we suspected that this may be associated with using suboptimal (fixed) exponents for the allometric scaling of the flow and volume parameters, this could not be verified due to the limited sample size of the study and concurrent uncertainty associated with estimation of alemtuzumab-specific allometric scaling exponents.[44] Finally, validation of our model in an independent patient cohort could have provided additional information on the reliability of its parameterization, its predictions and the conclusions drawn. However, because of the limited number of available PK profiles, particularly in the first 4 weeks, application of all PK data for the model development, as opposed to, for instance, a data-splitting approach for model development and validation, was deemed most likely to yield a trustworthy model.

5 | CONCLUSIONS

In conclusion, alemtuzumab induction therapy is associated with substantial between-subject PK variability in kidney transplant recipients. Part of this variability is explained by variability in body size, with lightweight individuals showing extended exposure to lympholytic alemtuzumab concentrations as compared to their heavier peers. LBW-adjusted dosing can be applied to correct for this phenomenon, showing potential as a marker to reduce between-subject variability in alemtuzumab exposure. Follow-up studies on the relation between alemtuzumab PK, lymphocyte dynamics and clinical outcomes are warranted to further substantiate the clinical potential and rationale for personalized alemtuzumab therapy in kidney transplantation.

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COMPETING INTERESTS

Tom Zwart, Suzanne Bezstarosti, Federica Achini, Marlies Reinders, Marco Schilham, Sebastiaan Heidt, Henk-Jan Guchelaar, Johan de Fijter and Dirk Jan Moes declare that they have no conflicts of interest that are directly relevant to the content of this article.

CONTRIBUTORS

T.C.Z., M.E.J.R., H.J.G., J.W.dF. and D.J.A.R.M. contributed to the study conception and design. S.H., S.B., M.W.S. and F.R.A. contributed data and analytical tools. T.C.Z. and D.J.A.R.M. performed the population pharmacokinetic analyses. The initial draft of the manuscript was written by T.C.Z. and D.J.A.R.M., and all authors commented on previous versions of the manuscript. All authors read and approved the final version of the manuscript.

DATA AVAILABILITY STATEMENT

The datasets generated during and/or analysed during this study are available from the corresponding author upon reasonable request. The NONMEM code used to generate and/or analyse the data described in this study is provided in the Supporting Information.

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SUPPORTING INFORMATION

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

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