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ORIGINAL PAPER

Population pharmacokinetics–pharmacodynamics of oral everolimus in patients with seizures associated with tuberous sclerosis complex

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Abstract

Everolimus is approved in Europe and in the USA for the adjunctive treatment of patients aged 2 years and older whose refractory partial-onset seizures, with or without secondary generalization, are associated with tuberous sclerosis complex. The objective of this analysis was to establish a population pharmacokinetic (PK)/pharmacodynamic model describing the relationship between seizure frequency and everolimus exposure to confirm the recommended target concentration range of 5–15 ng/mL. The PK model was a two-compartment model with first order absorption and clearance. CYP3A and P-gp inducers and body-surface area were shown to impact everolimus exposure, justifying dose adjustments. A Poisson distribution was found to adequately describe the random nature of daily seizure counts during the screening phase. A placebo effect on the Poisson seizure mean was implemented as an asymptotic exponential function of time leading to a new steady-state seizure mean. The everolimus effect was implemented as an inhibitory E_{max} function of C_{min} on the seizure mean, where E_{max} exhibited an asymptotic exponential increase over time to a higher steady-state value. Increasing age was found to decrease the baseline seizure mean and to prolong the half-life of the increase in E_{max}. The dependence of seizure frequencies on C_{min} was explored by simulation. The responder rate increased with increasing C_{min}. As C_{min} decreased below 5 ng/mL, variability in response became larger and responder rates decreased more rapidly. The results supported the recommended target concentration range for everolimus of 5–15 ng/mL to ensure treatment efficacy.

Keywords Population PK/PD · Non-linear mixed effect models · Count data · Tuberous sclerosis complex · Everolimus · Seizures

Introduction

Everolimus was first marketed for prevention of rejection of transplanted kidneys. It is also marketed for various oncology indications, including in Europe and in the US for the adjunctive treatment of patients aged 2 years and older whose refractory partial-onset seizures, with or without secondary generalization, are associated with tuberous sclerosis complex (TSC). Everolimus is a signal transduction inhibitor targeting the mammalian target of rapamycin (mTOR), or more specifically, mTORC1 (mTOR Complex 1). mTOR is a key serine–threonine kinase playing a central role in the regulation of cell growth, proliferation, and survival [1, 2]. The regulation of mTORC1 signaling is complex, being modulated by mitogens, growth factors, energy, and nutrient availability. mTORC1 is an essential regulator of global protein synthesis downstream on the PI3K/AKT/mTOR pathway, which is deregulated in the majority of human cancers. Consistent with the known activity of mTORC1, its inhibition by everolimus has been shown to reduce cell proliferation, glycolysis, and angiogenesis in solid tumors in vivo, both through direct

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antitumor cell activity and inhibition of the tumor stromal compartment.

Three clinical studies (EXIST program) evaluated the efficacy of everolimus in several indications associated with TSC [3–5]. Based on the results of the pivotal EXIST-3 study [3] (a randomized, placebo- and concentration-controlled trial), everolimus has been approved as a treatment for patients with refractory partial-onset seizures associated with TSC by the European Medicine Agency and the Food and Drug Administration. The current recommended target C_{min} range of 5–15 ng/mL for the treatment of seizures was based on efficacy and safety analyses derived from of the phase 3 studies EXIST-1, EXIST-2 and EXIST-3.

In the EXIST-3 study, both everolimus arms, one targeting low target exposure (LE), 3–7 ng/mL, and the other high target exposure (HE), 9–15 ng/mL, were superior to placebo. The HE arm was numerically better than the LE arm for efficacy, but not statistically significantly so. In spite of therapeutic drug monitoring and titration steps, many patients randomized to the HE arm had an observed C_{min} below the target range of 9–15 ng/mL during the 12-week maintenance period. However, exposure–efficacy analyses did establish C_{min} as a significant predictor of efficacy response. The positive exposure–efficacy relationship was established using regression models: linear regression to model post-baseline seizure frequency, and logistic regression for the responder rate [3].

These statistical regression models were useful to infer the existence of a non-null relationship between exposure and efficacy and of an additional effect of time on treatment. However, they were generalized linear models for time-averaged responses with simple linear dependencies on time-averaged C_{min} and, in the repeated-measures analysis, on a separate main effect of time on treatment. As such, they were unable to fully characterize: (i) the time-dependence of the relationship between exposure and response, (ii) the between-patient variability in the response, including any possible placebo effect, (iii) and the functional shape of the relationship between exposure and response. They also did not describe the within-subject variability of the baseline and post-baseline daily seizure counts.

Therefore, the work described herein was undertaken to develop a detailed pharmacokinetic/pharmacodynamic (PK/PD) model relating daily exposures to daily seizure counts, using flexible model forms for the joint dependence of exposure and time, and accounting for placebo effects and inter-patient variability. The model was qualified for its ability to predict seizure counts, and then it was used to confirm the recommended target concentration range of 5–15 ng/mL by means of simulation. Count-data models [6–10] were used to characterize and simulate the discrete and variable nature of the daily seizure counts.

Methods

This analysis was performed in three steps. First, a population PK (PopPK) model was built using all data available from patients enrolled in the EXIST-1, EXIST-2 and EXIST-3 studies. Then, a PopPK/PD model describing the seizure counts was built using EXIST-3 data, the only study with daily seizure data available. The exposure inputs into the PK/PD model were individual daily C_{min} values estimated from the PopPK model. Finally, simulations were performed to illustrate the everolimus and placebo exposure–efficacy relationship after 6 months of treatment.

Study designs

EXIST-1 was a phase 3 study evaluating treatment with oral everolimus at trough levels of 5–15 ng/mL *vs* placebo in 111 adults and pediatric patients with subependymal giant cell astrocytoma associated with TSC [4, 11].

EXIST-2 was a phase 3 study evaluating treatment with oral everolimus 10 mg daily in 65 adult patients with renal angiomyolipoma associated with either TSC or sporadic lymphangioleiomyomatosis [5].

In both EXIST-1 and EXIST-2, the primary outcome was based on reduction in tumor volume. EXIST-3 was a study in 366 patients with treatment-refractory partial onset seizures associated with TSC. 80% of the patients were children. The focus in EXIST-3 was reduction in seizure frequency.

The EXIST-3 study was divided into three phases: screening, core, and extension. The screening phase (also called baseline phase) lasted 8 weeks from screening to randomization. At randomization patients were assigned to one of the three treatment arms: placebo, everolimus LE, with C_{min} targeted between 3 and 7 ng/mL, or everolimus HE, with C_{min} targeted between 9 and 15 ng/mL. Included patients were to keep a stable antiepileptic drug (AED) regimen 2 months prior to the randomization date and until the end of the core phase. The core phase was divided into two periods: 6 weeks of titration, followed by 12 weeks of maintenance, during which doses could also be adjusted as needed. Patients were then offered to continue treatment in the extension phase, in which all patients received everolimus, including those patients originally randomized to the placebo arm [3]. Trough and peak PK samples were collected in all three studies, and a full PK profile was sampled in a subset of patients in EXIST-1.

General approach to nonlinear mixed-effects modeling

PopPK and PopPK/PD models were developed using techniques of nonlinear mixed-effects analysis [12–15]. Such a model consists of a structural model that describes general trends in the relationship between the response and predictor variables, and statistical models that describe inter- and intra-individual variability.

For PopPK modeling, population parameters were estimated by the maximum likelihood method as implemented in the first order conditional estimation method with interaction of NONMEM 7.2 [16]. Sequential PopPK/PD model fitting and simulations were performed in Monolix Suite 2016R1 using the SAEM algorithm for fitting [17]. Summaries of data and outputs were prepared with R Version 3.2.3. Of note, C_{min} predictions made by Monolix used the structural model equations and estimated individual PK parameters from the final PK model in order to get the same PK predictions regardless of the software used. Additional details can be found in Appendix 5. An exhaustive list of covariates used in this analysis can be found in Appendix 1.

Population PK modeling

Six hundred and thirteen PK observations and 8 patients (75 observations) were excluded: patients with no PK observations recorded, outliers or inconsistent records (e.g., trough concentration higher than peak concentration), concentrations below the limit of quantification (< 10% of the observations), and all nonzero PK concentrations for which the previous dose information (amount or time) was missing.

The everolimus PopPK model was updated based on a published model from the transplant indication of everolimus [18], on a non-published model based on EXIST-1, and on emerging data from the three EXIST studies. NONMEM library model ADVAN 4 TRANS 4 was used. Covariate effects that were assessed included age, body-surface area, race, sex, height, weight, and comedications (inducers of cytochrome P450 3A and P-gp).

Population PK/PD modeling

PopPK/PD modeling of the effects of everolimus on refractory partial-onset seizures associated with TSC aimed at assessing the efficacy of everolimus when C_{min} is within the 5–15 ng/mL range.

The PopPK/PD model was developed in a stepwise manner: first, a baseline model was developed using the screening phase data for all patients. Next, post-randomization data from patients on placebo was added, and the model was updated with a placebo-effect component. Finally, post-randomization data from patients on everolimus was added, and the model was updated with an effect of everolimus C_{min}.

This stepwise construction was based on the following assumptions:

- Absent any intervention, a stable disease condition ensues. That is, the within- and between-patient variability in daily seizure counts can be described by a model with no effects that depend on time, although between-subject variability may be influenced by covariates such as comedication use or demographics.
- The model describing this steady-state condition may be characterized using data from patients in the screening phase.
- Participation in the clinical trial affects the steady-state condition by causing the mean daily seizure count to change over time after randomization (placebo effect). This effect may be inferred by examining the behavior of patients in the placebo arm of the trial.
- Receiving everolimus treatment induces a further change on the daily seizure mean as a decreasing function of drug exposure as quantified by C_{min}.

The assumption of a steady-state disease condition may be unrealistic, but given the time frame of the clinical trial it would be difficult to distinguish a pre-treatment pattern of disease progression from any change induced by onset of treatment in the placebo arm. It is thought that the screening phase determines a snapshot of each patient's disease state at the start of the trial, which then serves as the initial condition for disease evolution according to placebo or everolimus exposure.

Baseline model

The baseline disease model, or screening phase model, describes the daily variation in seizure count prior to treatment with either placebo or everolimus, during the screening phase. A Poisson model and its derivatives were considered to describe the within-individual variability of observed daily seizure counts. An observation Y of a daily seizure count for an individual i is said to follow a Poisson distribution when the probability that it takes values $n = 0, 1, 2,\ldots$ can be expressed as:

$$P(Y_i = n) = \frac{\lambda_i^n}{n!} \cdot e^{-\lambda_i},$$

where λ_i is a positive individual parameter and ! the factorial function. The individual parameter λ_i represents both the mean and, as a special property of the Poisson distribution, the variance of the seizure count for the ith patient.

For the baseline model as well as for the subsequent placebo and everolimus models, the individual parameters λ_i were tested for having either normal or log-normal distributions.

After definition of the distribution driving the baseline seizure count, the influence of several covariates on the model parameters was assessed. Covariate values at the beginning of the screening phase were considered as influencing the baseline disease model. First, age and body surface area were considered, because there was a prior expectation that younger patients suffer more seizures. Next, race, sex and comedications (AEDs) were considered (for a list of AEDs see Appendix 1).

Placebo model

The placebo effect ($E_{placebo,i,d}$) was then modeled as a function of day, d, since initiation of treatment applied on λ_i by multiplication:

$$\lambda_{i,d} = \lambda_i \times E_{placebo,i,d}.$$

It was developed using data from all patients before the first administration of everolimus, i.e., from the screening phase for patients assigned to everolimus, and from the screening and core phases for patients assigned to placebo. In this step, population parameters for the baseline model were fixed to their estimated values. Population parameters for the placebo model and all individual parameters were estimated.

Several functions of d for $E_{placebo,i,d}$, such as linear, exponential, or asymptotic were tested to account for the placebo effect. Within an individual, $E_{placebo,i,d}$ was allowed to either increase or decrease with d.

Everolimus model

Finally, drug effect ($E_{everolimus,i,d}$) was assessed as the influence of C_{min} on the daily mean seizure count:

$$\lambda_{i,d} = \lambda_i \times E_{placebo,i,d} \times E_{everolimus,i,d}.$$

For each day where a seizure count was recorded, individual C_{min} was predicted by Monolix using the PopPK model as input into $E_{everolimus,i,d}$. To do so, individual parameters estimated in the PopPK modeling step were used, along with all the dosing information recorded during the study EXIST-3. For this reason, seizure count data from a patient after initiation of everolimus dosing was included only if that patient had PK data allowing estimation of patient-specific PK parameters.

For the sake of run-time, individual parameters from the baseline model for all patients and individual parameters from the placebo model for patients in the placebo arm were held fixed as they were already estimated in the previous steps. However, the individual parameters from the placebo model for patients in the everolimus arms were estimated using fixed population parameters. A final validation was performed by estimating all parameters of the final model simultaneously.

The following dependencies of $E_{everolimus,i,d}$ on C_{min} were considered: linear decreasing, inhibitory E_{max}, and asymptotic exponentially decreasing. Time effects were also tested to allow possible changes in the everolimus effect over time.

Finally, correlations between individual parameters and covariate effects were assessed. Covariate values at the first everolimus administration were considered as influencing the everolimus effect.

Model evaluation

Model evaluation for the PopPK/PD model was based mainly on simulation methods. Simulated and observed seizure counts were compared by visual predictive checks (VPCs) generated by Monolix using Monte Carlo simulations with the final parameter estimates. More details on the simulation methods and settings can be found in Appendix 2. The model was considered as adequate to describe the PD data in patients with seizures if the observed proportions were largely within the 5th–95th percentiles of the simulations.

Convergence assessment was also performed to explore the model stability (i.e., to ensure a global minimum). First, convergence graphs showing the value of parameters and objective function value at each iteration of the algorithm were evaluated to search for drastic changes in the estimated value, especially during the second step of estimation (k2 value in Monolix). Alternatively, no changes from the initial value may indicate an over-parametrization or mis-parametrization and need to be fixed. Finally, initial values of the population parameters were randomly changed and population parameters were re-estimated. Changes in the values of the estimates or in the objective function value may indicate an unstable model (i.e., local minimum) and suggest the need for changes in the structural or statistical model [19].

Exploration of the exposure–response relationship

The exposure–response relationship described by the final model was illustrated by a simulation study. This exercise allowed the prediction of the response for various exposures of everolimus for patients in EXIST-3, while correcting for the unexpected events occurring during a trial such as dropout or missing data. To assess whether the model supports the recommended C_{min} target range of

5–15 ng/mL, simulation input data sets were created consisting of patients from EXIST-3, with 41 separate data sets assigning constant C_{min} values during the core phase from 0 to 20 ng/mL in increments of 0.5 ng/mL. Each input data set included 28 days of a screening phase followed by 7 months of treatment.

From these input data sets, simulations generated 500 replicates of EXIST-3 at each C_{min} level. The final estimated individual parameters from the PopPK/PD model and the predefined C_{min} value were used as regressors to simulate each patient's daily seizure counts over time. These simulations were summarized as two sets of outputs. First, the predicted proportions of responders (> 50% reduction in seizure frequency from baseline) after 6 months on the various fixed values of C_{min} were determined and plotted. Second, weekly percent reductions from baseline in seizure frequency for selected scenarios (0, 3, 5, 10 and 15 ng/mL) were plotted to highlight the typical response associated with each exposure along with its variability. Seizure frequency was computed as the number of seizures divided by the number of days on which seizure information was known or simulated within the same period (baseline or weekly interval post baseline).

Results

Population PK model

The pooled PopPK dataset of everolimus contained 6649 concentrations from 531 subjects who participated in the EXIST-1, EXIST-2, and EXIST-3 studies. The observed concentrations are displayed in Fig. 1, and the demographics of the patients at baseline are described in Appendix 1.

The PK data was best described by a structural two compartment model including five main parameters: the first-order absorption constant (*ka*), the clearance (*Cl*), the intercompartmental clearance (*Q*), and the volumes of distribution of the central and peripheral compartments (respectively V_2 and V_3). As no data from intravenous dosing was available for everolimus, the bioavailability *F* could not be estimated. Hence, parameters were estimated as apparent clearances (*Cl*/*F*, *Q*/*F*) and apparent volumes (V_2/F, V_3/F). All individual parameters were best described with log-normal distributions. V_3/F and *Q*/*F* were judged to be perfectly correlated; they shared a random effect with a parameter quantifying the ratio of their standard deviations. Estimated population parameters are shown in Table 1. Due to instability of the model with regards to *ka* estimation and its high standard error, *ka* was fixed to its previously estimated value.

The final model used time-varying BSA as a covariate on *Cl*/*F* and *Q*/*F*, time-varying CYP3A inducer status on *Cl*/*F*, and BSA on V_2/F and V_3/F. Four separate splines described the BSA effect on clearances and volumes, each using the same two knots which were estimated to be BSA = 1.39 m^2 and 1.54 m^2. The first part of each spline up to the first knot was allometric, meaning the parameter was proportional to BSA raised to an exponent. The two clearances were modeled as having a common exponent of BSA, 0.609, and the two volumes were also modeled to

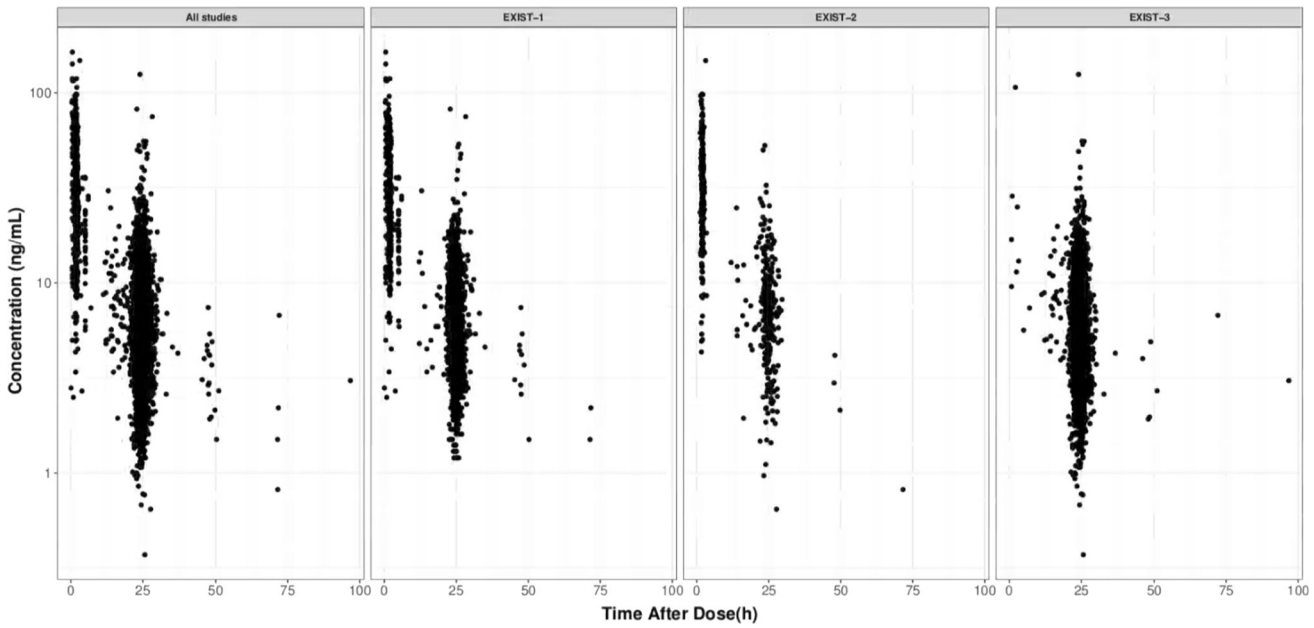

Fig. 1 Everolimus concentration plotted against time after dose for all studies and stratified by study

Table 1 Population PK parameter estimates

Parameter names	Parameter symbol (*) indicates a mu-parametrized parameter	Units	Pooled dataset (ka fixed)	
			Estimate	RSE (%)
Clearance	Cl (*)	L/h	20.0	2.3
Volume of central compartment	V_2 (*)	L	219.0	4.3
Intercompartmental clearance	Q (*)	L/h	15.4	9.3
Volume of peripheral compartment	V_3 (*)	L	335.7	11.0
Absorption rate	ka (*)	h^{-1}	10.8	Fixed
BSA effect on Cl and Q	$\beta_{BSA\text{-}Cl,Q}$		0.609	6.0
BSA effect on volumes	β_{BSA-V_2,V_3}		0.494	16.1
First knot	*Knot* 1 (*)	m^2	1.390	2.72
Distance between knots	Ln(knot 2) − ln(knot 1)	$Ln(m^2)$	0.1	Fixed
Cl ratio for inducer (*)			1.1	0.90
sd ratio for V_3 to Q			3.5	11.2
Random effect parameters	ω_{Cl} variance-based shrinkage (%)		0.409 (8)	11.2
	ω_{V_2} variance-based shrinkage (%)		0.475 (19)	19.1
	ω_Q variance-based shrinkage (%)		0.455 (26)	23.2
	cor_{Cl,V_2}		0.912	14.3
Residual error parameters	σ_{prop} variance-based shrinkage (%)		0.342 (5)	3.2

Relative standard errors were computed as $100 * \frac{SE}{|estimate|}$, unless parameter was mu-parametrized (*) in which case the formula $100 * \sqrt{e^{SE^2} - 1}$ was used, SE being the standard error computed by NONMEM

have another common exponent of BSA, 0.494. Between the first and the second knots, the four splines then were quadratic in ln(BSA), and then beyond the second knots they were constant at parameter values typical of adults. With the presence of CYP3A or P-gp enzyme inducers, the corresponding apparent clearances (L/h) and normalized clearances ($L/h/m^2$) increased by 10%.

Sensitivity analyses were performed to assess the impact of several factors on the parameter estimates: inclusion or exclusion of outliers, and increased or decreased fixed *ka* value; see Appendix 2. No modifications were found to improve the model fit. Goodness-of-fit plots and bootstrapping results are also shown in Appendix 2.

The model was further qualified for its ability to estimate individual values of C_{min}, the exposure inputs into the PopPK/PD model, by more detailed assessment of residuals. Differences between observed and predicted C_{min} values are summarized in Appendix 2 Fig. 2. Note from Table 1 that the epsilon-shrinkage was only 5%, so that such residuals-based diagnostics are credible. Overall and for each subgroup, the mean differences are centered around 0. These results demonstrate the adequacy of the model to predict everolimus trough concentrations for patients in the three studies, in particular for EXIST-3, where the PopPK model's C_{min} values were used as inputs in the PopPK/PD model.

Population PK/PD model

The PopPK/PD analysis dataset contained 163,963 daily seizure observations and 126,202 doses arising from 366 patients who participated in EXIST-3. Figure 2 summarizes the observations by showing the number and proportion of observations in each seizure category (0, 1, 2, 3 and 4, > 4 seizures/day) versus time stratified by analysis phase. The figure suggests the presence of a placebo effect as well as an everolimus effect. During the screening phase (left plot), 29.0–30.7% of the observations were zero seizures/day. After randomization, in the placebo arm (middle plot), an average of 34.9% of the observations were zero seizures/day during the interval of weeks 16–18; and in the everolimus arm the proportion of days without seizures increased to 46.4% during the same interval: the effect of everolimus seemed larger than the placebo effect. Similarly, the proportion of days without seizures kept increasing after the core phase, due to the transition of patients in the placebo arm to everolimus and the continuing efficacy of everolimus.

Baseline model

In the screening phase 21,818 daily seizure counts were recorded in 366 patients. Whether the seizure count data follows a Poisson distribution was evaluated by comparing

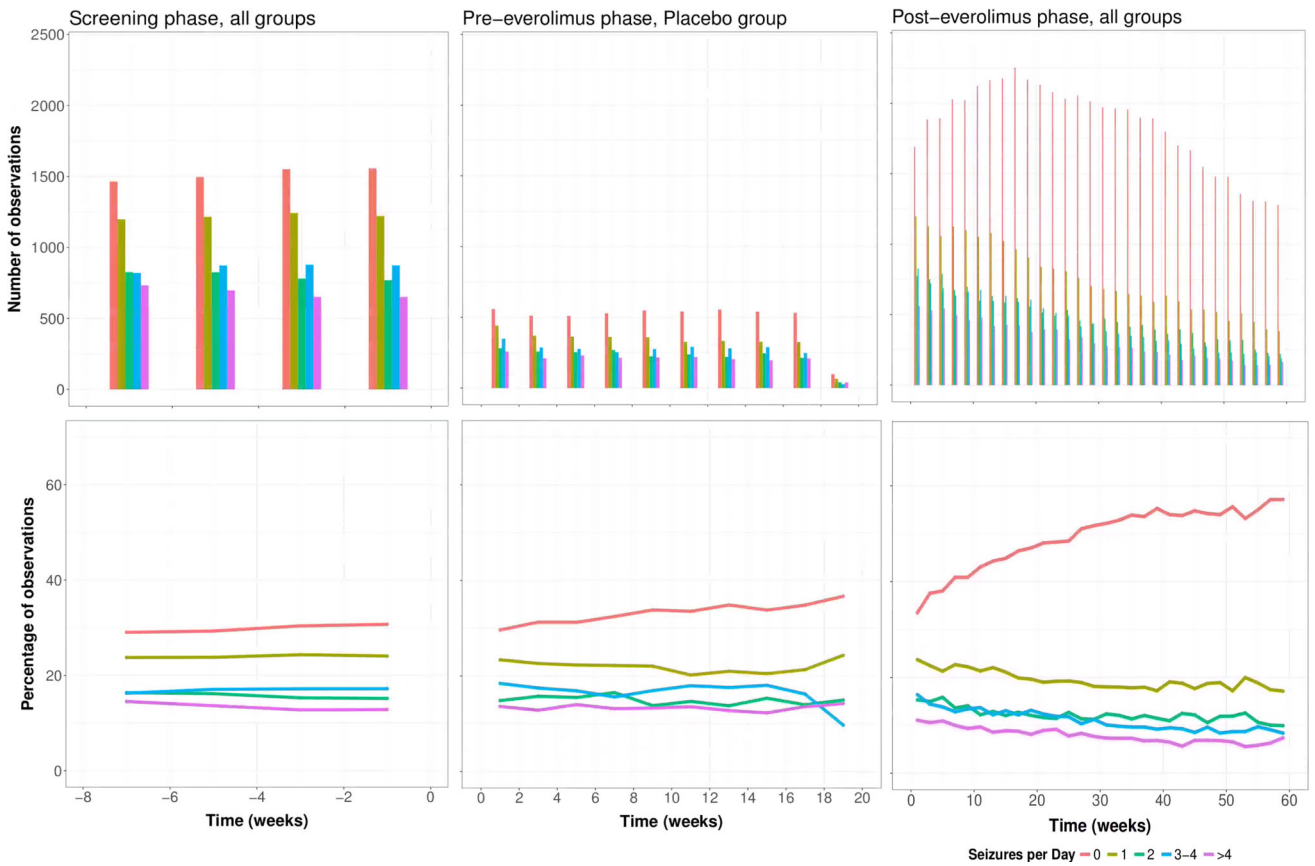

Fig. 2 Proportion and number of observations in each number of seizures category (0, 1, 2, 3 and 4, > 4 seizures/day) versus time, observed in EXIST-3 and stratified by analysis phases. Data was binned every 2 weeks. Percentage of observations (bottom plots) in each category was computed relative to the total number of observations (top plots) in the corresponding time bin

individual means and variances in Fig. 3. A Poisson distribution relies on the assumption that for each individual, the mean of the daily seizures counts over the screening phase equals their standard deviation. There was no marked deviation from the Poisson distribution's expected equality of the individual mean and variance. Therefore, the daily number of seizures during the screening phase (i.e., the assumed baseline steady-state disease condition) was described by a count model based on a Poisson distribution.

Then, the effect of age or BSA on baseline disease model parameters was assessed. Addition of the log-transformed and centered age effect on λ decreased the objective function value by 26 points and was the first most significant covariate kept in the model. A Wald test performed by Monolix confirmed the covariate effect. Additionally, inclusion of the covariate decreased the inter-individual variability. In a second step, other covariate effects were tested: sex, race, co-treatments (AEDs). No other covariates were significant enough to be added in the model. In particular, no AED effects were retained in the model. The model for individual Poisson mean daily seizure count determined from the screening-phase data was thus

$$\log(\lambda_i) = \log(\lambda_{pop}) + \beta_{\lambda-AGE0} \times [\log(AGE0_i) - \log(AGE0_{median})] + \eta_{\lambda,i},$$

where λ_{pop} and $\beta_{\lambda\text{-}AGE0}$ were parameters and $\eta_{\lambda,i}$ was a random effect. Table 2 summarizes the final parameter estimates for the baseline disease model along with the results of the subsequent placebo and everolimus models. Of note, the estimate for λ_{pop} is 1.45, close to the observed medians of individual mean and standard deviation of the seizure counts as shown in Fig. 3.

Placebo model

The placebo effect ($E_{placebo,i,d}$) was then modeled by adding the placebo group in the analysis dataset. The size of the augmented data set was now 36,815 observations from 366 patients (daily seizures at baseline from all 366 patients plus daily seizures from the core phase for the patients treated with placebo only). An asymptotic exponential time effect model was selected for subject i as:

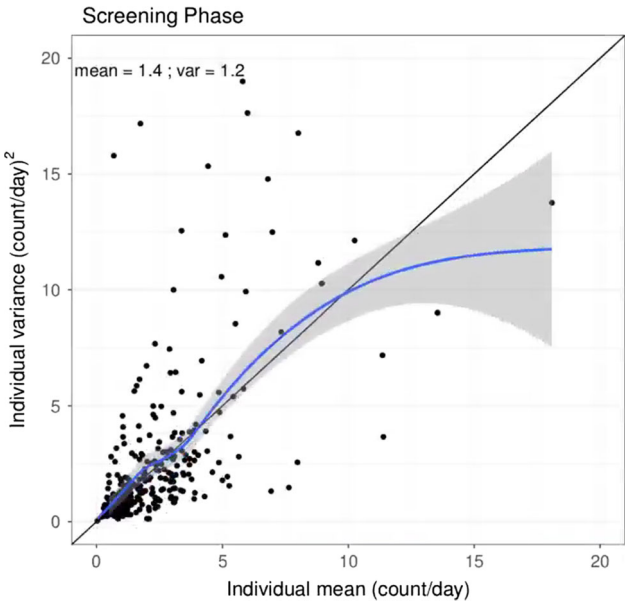

Fig. 3 Dispersion plots for screening phase. Each dot represents the mean and the variance of the daily seizure counts for an individual. The blue line represents a smooth regression and the grey area a confidence interval around the smooth line. The annotated "mean" and "var" are median values of the individual means and variances (Color figure online)

$$\lambda_{i,d} = \lambda_i \times E_{placebo,i,d}$$

with

$$E_{placebo,i,d} = 1 + \left(Max_{PCB,i} - 1\right) * \left(1 - e^{-Slope_{PCB,i} * (d - Tlag_i)}\right)$$

and $E_{placebo,i,d} = 1$ before the randomization, d being the day of the observation, $Tlag_i$ being the day of the randomization (end of screening phase), and $Max_{PCB,i}$ and $Slope_{PCB,i}$ two parameters characterizing respectively the maximum of the placebo effect and a log-slope of the time-dependent change of the placebo effect. The estimated value of $Slope_{PCB}$ corresponds to a half-life of 18.3 weeks (95% CI 11.2–44.5 weeks) for a typical patient (population value). Of note, $Max_{PCB,i}$ varied among patients with a log-normal distribution with median 0.5. The individual value directs the orientation of the placebo effect: below 1 (322 patients), the placebo effect leads to a decrease of the mean number of daily seizures over time, and above 1 (44 patients) describes an increase of this value.

Everolimus model

An everolimus effect ($E_{everolimus,i,d}$) was then modeled by adding the patients receiving everolimus (everolimus LE and HE arms and placebo patients who received everolimus during the extension phase) in the full analysis dataset. The final model was an inhibitory E_{max} model where the maximal inhibition increased over time as an asymptotic exponential function to 1; smaller values than 1 for this asymptotic value of E_{max} were tested but not selected. This asymptotic exponential equation was driven by the time since the first everolimus administration, $Tlag_{EVE,i}$, and an estimated parameter $SlopeTime_{Emax,i}$, describing the log-slope of E_{max} increase. Exponential random effects were applied to these parameters. The final structural model for the placebo and everolimus effects was described by:

$$\lambda_{i,d} = \lambda_i \times E_{placebo,i,d} \times E_{everolimus,i,d}$$

with

$$E_{everolimus,i,d} = 1 - Emax_{EVE,i,d} \times Cmin_{i,d} / \left(Cmin_{i,d} + C_{50,i}\right)$$

and

Table 2 Summary of PopPK/PD parameter estimates and Monolix relative standard error (RSE%) for the baseline disease model, the placebo model, and the everolimus effect model

Parameter names	Parameter symbols	Units	Estimate	RSE%
Mean number of seizures at baseline	λ_{pop}	Seizures/day	1.45	5
Age effect on λ_{pop}	$\beta_{\lambda\text{-}AGE0}$		− 0.347	19
Maximum placebo effect	Max_{PCB}		0.50	20
Log-slope of time-dependent placebo effect	$Slope_{PCB}$	h^{-1}	2.26×10^{-4}	31
Concentration leading to 50% of everolimus effect	C_{50}	ng/mL	1.77	1
Log-slope of everolimus effect onset	$SlopeTime_{Emax}$	h^{-1}	3.38×10^{-5}	29
Age effect on everolimus effect	$\beta_{SlopeTime_{Emax}-AGE1}$		− 1.05	36
Random effect parameters	ω_{λ} variance-based shrinkage (%)		0.94 (2.8)	4
	$\omega_{Max_{PCB}}$ variance-based shrinkage (%)		1.500 (52)	12
	$\omega_{Slope_{PCB}}$ variance-based shrinkage (%)		2.08 (70)	14
	$\omega_{SlopeTime_{Emax}}$ variance-based shrinkage (%)		4.27 (53)	6

$$Emax_{EVE,i,d} = 1 - e^{-SlopeTime_{Emax,i} \times (d - Tlag_{EVE,i})}$$

$d\text{-}Tlag_{EVE,i}$ was set to 0 before the first everolimus administration.

Sex, age, BSA and race effects were tested in a forward-selection process on $SlopeTime_{Emax}$. With the addition of the first covariate, age (on the log scale), the objective function value significantly decreased by 142, and the standard deviation of the random effect for $SlopeTime_{Emax}$ decreased from 4.54 to 4.27. A significant Wald-test result (p value = 0.0049) confirmed the statistical significance of age as a covariate. After the first everolimus administration, $Emax_{EVE,i,d}$ increased up to 1 with a half-life of 122.1 weeks (95% CI 83.2–396.7 weeks) for a typical patient of 10.1 years (based on the median age at start of everolimus and on the population estimate). This is a long typical half-life, but the inter-individual standard deviation was estimated to be 4.27. One standard deviation shorter is only 1.71 weeks. A change in age from the median value of 10.1 years to 6 or 18 years would modify the half-life of $Emax_{EVE,i,d}$ from 122.1 weeks to respectively 70.7 and 224 weeks. Inclusion of a second covariate did not improve the model fit. Final parameter estimates are summarized in Table 2.

To assess the ability of the final model with covariates to describe the data, results of VPCs were plotted in Fig. 4 (with focus on the screening and core phase on the top row, and the entire data on the bottom) to show the agreement between the observed proportion of observations at each time in each category (lines) and the 90% prediction intervals for those proportions (grey areas). A generally good agreement between observations and predictions can be seen. Further validations in Appendix 2 also support the stability and selection of the final model. Additionally, a final run was performed with estimation of all parameters, with similar parameters estimated. The final codes of each model can be found in Appendix 3. Consequently, this final model was used for simulation to illustrate the exposure–response relationship.

Exposure–response relationship

Figure 5 shows the 5th, 50th and 95th percentiles (prediction interval) of the proportions of responders after 6 months of treatment versus C_{min} as determined by simulations of the model. With C_{min} concentrations above 5 ng/mL, the median expected response rate ranges from 30 to 50%. The median proportion of responders is positively correlated with C_{min}. The curve depicting the median proportion of responders appears approximately piecewise linear, with a steeper slope before 5 ng/mL, suggesting an advantage to targeting C_{min} values of 5 ng/mL or greater.

A concentration of 3 ng/mL was associated with a slightly lower median proportion of responders than 5 ng/mL, but the uncertainty, represented by the 90% prediction interval, was large. Additional support for the clinical superiority of 5 ng/mL over 3 ng/mL is found by considering Fig. 6, which shows how median seizure frequency is

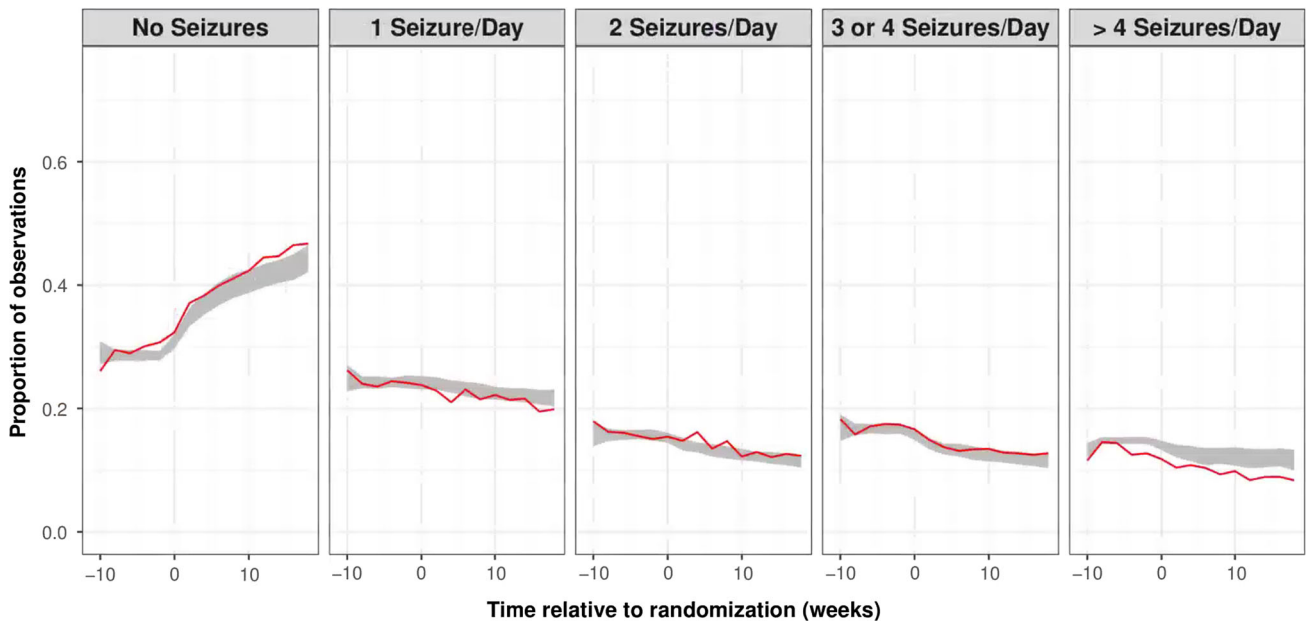

Fig. 4 Observed and simulated proportions of observations in each category (0, 1, 2, 3 or 4, and > 4 seizures/day) plotted against time. The red lines show the observed proportions of observations versus time in the different categories. The grey areas show 90% prediction intervals of the proportions based on simulations of the model. Time has been transformed as "time relative to randomization" for clarity purposes, where time = 0 is the time of randomization for each patient (Color figure online)

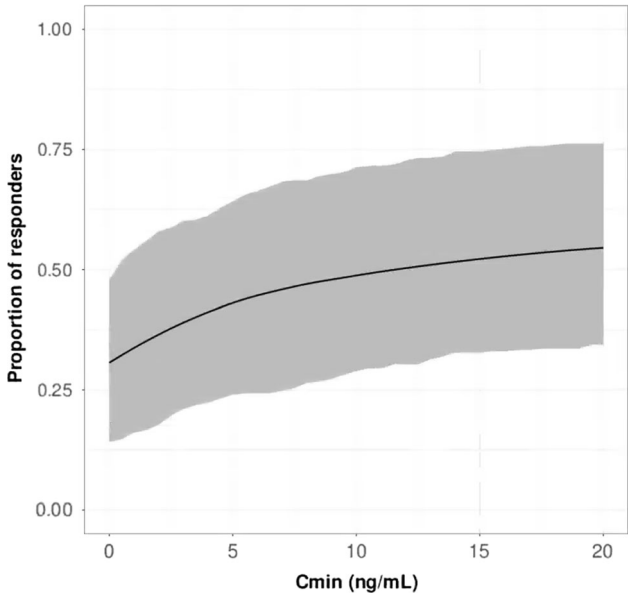

Fig. 5 Predicted proportion of responders after 6 months of treatment at constant C_{min}. The black line shows the median predicted proportion of responders versus C_{min} according to the model, and the gray area shows the middle 90% of such predicted proportions

predicted to continually decrease over time on treatment. However, a C_{min} of 3 ng/mL has a predicted probability 0.05 of an increase of 84.5% or more in seizure frequency at 6 months. On the other hand, for a C_{min} of 5 ng/mL the seizure-frequency increase corresponding to the 0.05 probability limit is 58.6%. With this in mind, it becomes clear that while some patients may respond already at 3 ng/mL, a concentration of at least 5 ng/mL should be recommended to avoid treatment escape and to increase the proportion of responders. Appendix 4 shows a version of Fig. 6 stratified by age for a selected group of C_{min} values. It can be seen that the relative advantage of 5 ng/mL over 3 ng/mL is consistent across age groups where it is shown that the 5th percentile of the predictions can reach values as low as − 100% reduction from baseline in seizure frequency (doubling the number of seizures from baseline) at a concentration of 3 ng/mL, versus − 50% at a C_{min} level of 5 ng/mL.

Discussion

Population PK

This analysis evaluated the dose, exposure, and exposure–response relationships of everolimus for patients with refractory partial-onset seizures associated with TSC. A PopPK model was first built to evaluate and then to predict daily C_{min} concentrations. BSA was found to be a significant covariate on clearances and volumes, and concomitant CYP3A or P-gp inducers on clearance. It is well known that everolimus is primarily eliminated by metabolism via cytochrome P450 enzymes and by P-gp transporters. The inducer effect found in the PopPK model confirms the earlier findings from drug–drug interaction analyses [20–25].

In addition, results from Kovarik et al. [18] also highlighted the influence of age-correlated covariates (height and weight) in the renal transplant indication, while this effect was not significant enough to induce a dose adaptation. Of note, Kovarik's analysis was based on a population of children above 16 years old and adults (median 44 years, and up to 70 years) whereas the youngest patient included in the EXIST studies was 1 year old (median 11 years, and up to 61 years). The larger age span in our dataset relative to Kovarik's may explain why BSA was kept in our model, leading to a recommended starting dose/BSA to try to get exposure into the target range quickly.

Using estimated individual parameters, relationships of clearance with age, BSA, and inducer status are illustrated in Table 3. The pattern of clearance across age and inducer groups supports dosing by mg/m^2 to best match PK exposure across age groups. Results showed that mean clearance (L/h), either associated or not with the use of inducers, was positively related with age. When correcting by BSA, the mean clearances were near 20 L/h/m^2 across all age × inducer groups except for adults without inducers where the mean was 13.66 L/h/m^2.

Previous unpublished analyses have reported larger effects, on the order of 22%, for the increase of everolimus clearance by inducers. Here, the effect seemed more modest, only around 10%. However, Table 3 reveals a pattern that reconciles the apparent discrepancy. Although the overall effect was only around 10%, this was a balance between virtually no effect for children < 18 years old, and an effect of about 30% for adults. As noted above, the age distribution here was shifted towards younger children relative to previous studies.

During this analysis, minimization was not successful until *ka* was fixed to the previously estimated value (cf Appendix 2). Nonetheless, the model was judged to be adequate for the newly enlarged pool of data. The model correctly captured the trough concentrations which were used as the exposure variable in the PopPK/PD analyses.

Finally, a sensitivity analysis was performed to evaluate the model reliability with regards to: influence of outliers, value of fixed ka, precision of parameters estimates (bootstrapping). These evaluations (cf Appendix 2) confirmed the stability of the PopPK final model.

As reported by French et al. [3], most patients randomized in the HE arm in the EXIST-3 study did not reach the anticipated exposure with a median C_{min} of 8.3 ng/mL at the end of the core phase. During the study's titration

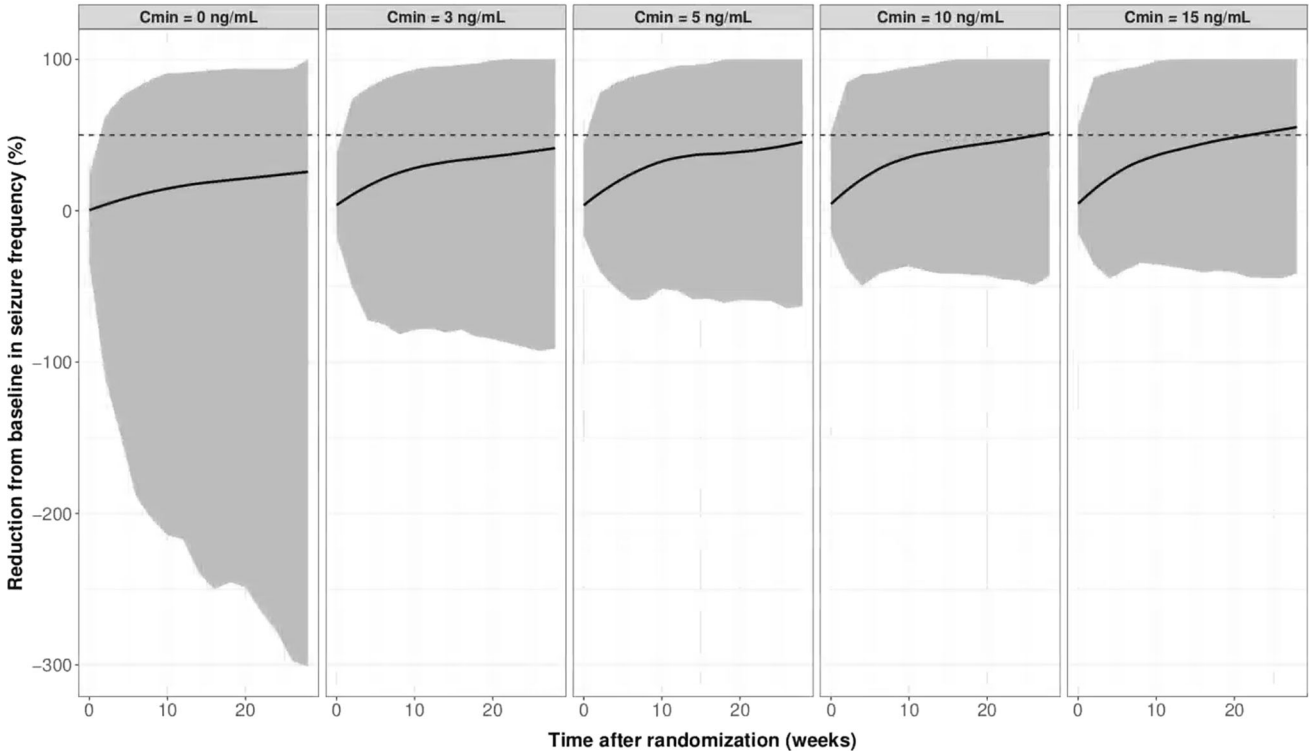

Fig. 6 Predicted percentage reduction from baseline in seizure frequency of responders for versus time for all patients, for C_{min} values of 0, 3, 5 10 and 15 ng/mL. In each panel, the black line shows the median predicted reduction from baseline in seizure frequency versus time according to the model, and the gray area shows the middle 90% of such predicted reductions. The dashed line represents the threshold of 50% reduction for responders. Note that a reduction is a positive value here, and a negative reduction represents an increase in seizure frequency

Table 3 Summary statistics of clearance and BSA-corrected clearance by age group and inducer status

Inducer status	Age groups	Number of observations	Mean clearance	CV% clearance	Mean BSA-corrected clearance	CV% BSA-corrected clearance
Units	Years		L/h	%	L/h/m^2	%
With inducer	< 3	32	12.36	24.30	22.19	34.78
	3 to < 6	263	15.70	30.90	21.12	31.54
	6 to < 12	465	22.22	31.27	22.17	32.75
	12 to < 18	470	27.50	34.09	18.21	43.20
	≥ 18	419	32.98	36.40	18.85	37.55
	All patients	1649	25.23	42.71	20.03	37.43
Without inducer	< 3	241	12.31	42.11	20.73	37.72
	3 to < 6	838	18.01	34.82	24.28	35.75
	6 to < 12	1591	21.28	37.76	20.63	36.34
	12 to < 18	1051	27.89	38.81	18.86	42.81
	≥ 18	1279	24.88	38.35	13.66	38.44
	All patients	5000	22.61	42.58	19.09	42.92

CV% coefficient of variation calculated as 100 * standard deviation/mean, *BSA* body-surface area

period, only three titration steps of everolimus 2 mg (no inducers) or 4 mg (with CYP inducers) were allowed. This might not have been sufficient enough to reach the HE target in patients with high clearance or high body surface area.

Population PK/PD

The effect of everolimus on patients with treatment-refractory partial-onset seizures associated with TSC was then evaluated by building an exposure–response model describing the relationship between C_{min} and the daily number of seizures observed in children from 1 to 18 years and in adults enrolled in EXIST-3. This model characterized several components such as the baseline disease effect, the placebo effect, and the everolimus effect.

Predicted C_{min} associated with a 50% reduction from baseline in seizure frequency justified the target range of 5–15 ng/mL in this indication. In particular, it was shown that at exposures below 5 ng/mL around 8.7% of patients are predicted to have *increases* in seizure frequency of more than 50%, after 6 months of treatment. A target concentration of at least 5 ng/mL was proposed to maximize the effect of everolimus as early as possible, on the largest population possible. It also confirms the necessity of dose adjustment based on C_{min} values and on the observed clinical response.

The median proportion of responders is positively correlated with C_{min} exposure and is close to plateau at or above C_{min} of 15 ng/mL. There are limited data in patients with C_{min} concentration values > 15 ng/mL in existing clinical studies and the safety profile with C_{min} exposure > 15 ng/mL is not well established, making 15 ng/mL an upper bound of the therapeutic range [3].

Due to the size of the dataset and the complexity in analyzing count data, a sequential (also called stepwise) modeling approach was used to build this model: first the baseline disease model, then the placebo effect, and then everolimus effect.

The everolimus and placebo effects were estimated assuming that the baseline disease was stable over time before and after the randomization date, which means that the model describing the baseline disease did not change after randomization. In particular, no disease progression independent of the placebo effect was modeled. The only factor deemed to significantly influence the baseline was the age at start of screening, where children tended to experience more seizures than adults.

All effects of time were attributed to either the placebo or everolimus effects. For placebo this effect would lead to a plateau. For everolimus, the time-dependence was on the E_{max} parameter, i.e., the maximal effect at large concentrations, which the model indicated would increase over time to a maximum of 100% reduction from baseline in seizure frequency. However, the typical time needed to reach this maximal effect was estimated as longer than the clinical trial lengths. The model is applicable only for the scale of times represented by the data; extrapolations to the predicted limiting behavior must be interpreted with caution.

The model also identified an effect of age on the everolimus effect. Time to reach everolimus maximal effect was predicted to increase with increasing age; everolimus, therefore, should attain its maximal effect faster in children than in adults.

Finally, the everolimus effect was described as an E_{max} function quantifying the effect of C_{min} on mean daily seizure count. A gradual decrease in seizure frequency was observed in EXIST-3 with no maximal decrease evident during the trial (but may be seen later in the extension data). The final model identifies three components to explain that gradual decrease of daily seizure counts. The first component is the placebo effect; for most patients, the placebo effect contributes to decreasing seizure counts. The second component is related to everolimus exposure, i.e., the time it takes to reach a steady-state C_{min} after dose titrations and PK accumulation. The third component is the increase of E_{max} over time.

It has been noted in several publications that seizure counts may be best described by a negative binomial distribution, and also include Markov elements showing the influence of the previous number of seizures on the current number of seizures [7, 10]. Our modeling exercise on data from EXIST-3 did not have similar conclusions. The data itself did not support such a model as (1) the dispersion plot presented in Fig. 3 did not show the high overdispersion as seen in earlier publications, and (2) serial correlation plots (data not shown) could not detect a specific correlation between current and previous counts. The difference between our results and those of previous studies may be due to the etiology of the seizures: in our study seizures were associated with TSC. In other studies, the etiology was not specified, but likely included patients without TSC.

Conclusions

A two-compartment disposition model with first order absorption and elimination properly described all PK data collected in both adults and children from the EXIST-1, EXIST-2 and EXIST-3 clinical trials. Body surface area effects on clearances and volumes were kept in the final model to account for differences between children and adults. The effect of inducers of CYP3A or P-gp on central compartment clearance was retained in the final PK model leading to an increase of apparent clearance by 10% overall but less in children and more in adults. The overall

variability for clearance (and therefore AUC) was reduced by BSA—normalization of clearance (L/h/m^2) compared to apparent clearance (L/h). This supported dosing by mg/m^2 to best match PK exposure across age groups.

A PopPK/PD model was then built using data on adults and children with seizures from EXIST-3. Covariates explained some variation in the seizure count at baseline and in the everolimus treatment effect on the seizure counts, with lower numbers of seizures for adults than for children, and a maximal treatment effect reached faster among children. In simulations with constant C_{min} concentrations at either 5 or 15 ng/mL, the median expected response rate was respectively around 30 and 50% at 6 months. Everolimus C_{min} concentrations below 5 ng/mL were associated with a larger variability of response, leading to an increase in non-responders. These results emphasized the importance of a target C_{min} range of 5–15 ng/mL to insure efficacy of treatment as well as a tolerable safety profile. The exposure–response profile depicted by the model highlighted a high inter-individual variability in the C_{min}–response relationship, confirming the need for close monitoring of the concentrations.

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Compliance with ethical standards

Conflict of interest All authors are or were employees of Novartis at the time of the research.

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