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ARTICLE

Model-Informed Drug Development for Everolimus Dosing Selection in Pediatric Infant Patients

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Everolimus is currently approved in Europe as an adjunctive therapy for patients aged ≥ 2 years with tuberous sclerosis complex (TSC)–associated treatment-refractory partial-onset seizures, based on the EXIST-3 study (NCT01713946) results. As TSC-associated seizures can also affect children aged between 6 months and 2 years, a modeling and simulation (M&S) approach was undertaken to extrapolate exposure (trough plasma concentration (C_{min})) after a dose of 6 mg/m^2 and reduction in seizure frequency (RSF). A physiologically based pharmacokinetic model using Simcyp was developed to predict C_{min} in adult and pediatric patients, which was then used by a population pharmacodynamic model and a linear mixed effect model to predict short-term and long-term efficacy in adults (for validation) and in children, respectively. Based on the results of the M&S study, everolimus at the dose of 6 mg/m^2 is anticipated to be an efficacious treatment in children 6 months to 2 years of age (up to 77.8% RSF) with concentrations within the recommended target range.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
☑ Patients aged ≥ 2 years affected by tuberous sclerosis complex (TSC)–associated treatment-refractory partial-onset seizures appear to benefit from treatment using everolimus.

WHAT QUESTION DID THIS STUDY ADDRESS?
☑ This study aimed to extrapolate the efficacy of everolimus in children younger than 2 years of age, as no efficacy data were available.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?
☑ This modeling and simulation (M&S) study predicts a clinically relevant reduction in the disease symptoms if the target population is treated with everolimus, with no increase in exposure beyond the upper limit of trough of 15 ng/mL.

HOW MIGHT THIS CHANGE DRUG DISCOVERY, DEVELOPMENT, AND/OR THERAPEUTICS?
☑ Children suffering with TSC seizures may benefit from a new therapeutic option. This exercise demonstrates the usefulness of the M&S study to provide helpful insights in pediatric development bypassing formal and challenging clinical trials in a vulnerable population.

Tuberous sclerosis complex (TSC) is a genetic disorder characterized by multiple benign tumors throughout the body. It is caused by mutations in either the *TSC1* or *TSC2* genes, resulting in constitutive overactivation of mammalian target of rapamycin.[1] TSC–associated partial-onset seizures (POS), reported in up to 90% of patients, are one of the most common presenting symptoms of TSC. The seizure semiology varies and can change throughout a patient's lifetime.[2] Approximately 60% of the patients remain treatment refractory.[3,4] Everolimus, a mammalian target of rapamycin inhibitor, achieves antitumor activity by inhibiting the phosphatidylinositol 3-kinase/protein kinase B/mammalian target of rapamycin pathway and by downregulating angiogenesis.5

Everolimus received approval in Europe for the adjunctive treatment of patients aged 2 years and older with TSC-associated refractory POS, with or without secondary generalization. This approval was based on the results of EXIST-3 (EXamining everolimus In a Study of Tuberous sclerosis, NCT01713946), a large phase III study in 294 pediatric patients aged 2–18 years with TSC-associated refractory POS,[6] along with data from two previous phase III studies, EXIST-1 (NCT00789828) and EXIST-2 (NCT00790400),[7,8] performed in patients with TSC. The starting dose of everolimus in patients < 6 years of age was 9 and 6 mg/m^2, respectively, with and without the concomitant administration of cytochrome P450 3A4 (CYP3A4) or phosphoglycoprotein (P-gp) inducers.

To inform dosing selection in patients younger than the age of 2 years, we conducted a modeling and simulation (M&S) study to estimate the exposure and efficacy (short term and long term) of everolimus as adjunctive treatment in patients aged > 2 years and extrapolate for patients with TSC-associated refractory POS who started everolimus at 6 months to 2 years of age. Although neonates may also suffer from epilepsy, these are part of a different indication,

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called infantile spasm. Everolimus is indicated in patients older than 2 years of age only for those seizures that are refractory. Therefore, a minimum age of 6 months was considered as the time to start with everolimus for those patients who do not respond to other existing and approved medications. The scope of this article concerns only the explanation of modeling methods pertaining to the efficacy of everolimus in the target population as no concerns of safety were made given the large knowledge available on everolimus in the target population. Of note, in clinical practice, everolimus concentrations will be monitored and the dose adjusted if necessary.

METHODS

Modeling strategy

The M&S framework consisted of the following three different models that were used in conjunction to enable the prediction of precise and reliable pharmacokinetic (PK) and pharmacodynamic (PD) markers in pediatrics:

- Prediction of daily exposure (trough plasma concentration (C_{min})) of everolimus in pediatric patients with TSC using a physiologically-based PK (PBPK) model
- Prediction of short-term efficacy (percentage reduction in seizure frequency [%RSF]) of everolimus in pediatric patients with TSC-associated POS
- Prediction of long-term efficacy (%RSF) of everolimus in pediatric patients with TSC-associated POS

The M&S framework was built in three steps, as described in **Figure 1**. First, three models were built and validated separately based on the observed PK and PD data from the EXIST-1, EXIST-2, and EXIST-3 studies. Then, the three models were combined and qualified by simulating the exposure and response of patients older than 2 years of age and by comparing the outcome with the observed data from the EXIST-3 study. In that step, exposure was simulated using the PBPK model and used as a regressor to drive the two PD models for short-term and long-term responses. Finally, exposure and efficacy in patients between 6 months and 2 years of age at initiation of everolimus treatment were extrapolated.

Data and populations

PK and PD data were extracted from the three EXIST trials. Study designs were already described in previous articles.[7,8] For exposure, everolimus concentrations from the pooled studies EXIST-1, EXIST-2, and EXIST-3, in which the youngest observed patient started everolimus at the age of ~1 year, were used. For efficacy, EXIST-3 (where the youngest observed patient started everolimus at the age of 2 years) was the only study in this indication that collected seizure data in daily patient-reported dairies. EXIST-3 was a phase III, three-arm, randomized, double-blind, placebo-controlled study comparing the efficacy and safety of once daily oral everolimus titrated to achieve a target trough level of 3–7 ng/mL (low target) or 9–15 ng/mL (high target) vs. matching placebo in patients 1–65 years of age with a clinical diagnosis of TSC. Therefore, based on the available data, the extrapolation was performed on two target populations:

1. Extrapolated exposure in patients who started everolimus at 6 months to 1 year of age
2. Extrapolated efficacy in patients who started everolimus at 6 months to 2 years of age

As the efficacy of a treatment for seizures is evaluated after several weeks of follow-up, the efficacy was simulated for 6 months (short term) and 24 months (long term) in patients who started everolimus.

Prediction of exposure

A PBPK model was built and qualified using the Simcyp simulation software (Certara LP, Sheffield, UK)[9-11] to predict everolimus exposure (C_{min}) in 200 patients aged between 6 months and 1 year using the following approach:

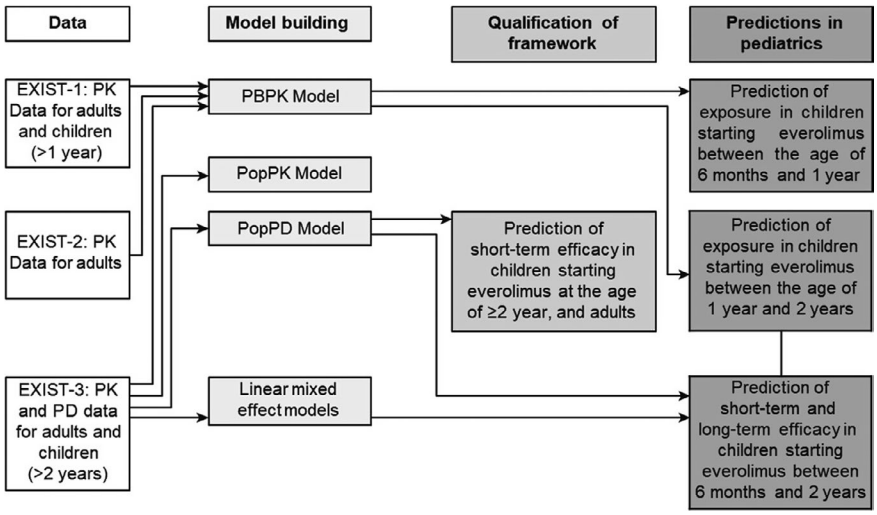

Figure 1 Workflow for modeling and simulation framework. EXIST-1, NCT00789828; EXIST-2, NCT00790400; EXIST-3, NCT01713946; PBPK, physiologically-based pharmacokinetic; PD, pharmacodynamics; PK, pharmacokinetics; PopPD, population PD; PopPK, population PK.

- An adult PBPK model was built to predict everolimus PK after single and multiple doses and interaction with CYP3A4 perpetrators to verify CYP3A4 contribution to clearance (**Supplementary Material S1**).
- This model was then qualified to predict PK in adult patients with TSC (18–62 years) from EXIST-1, EXIST-2, and EXIST-3.[6,12,13] The qualified adult model compound file was then used to predict PK in pediatric patients.
- The compound file was refined "top-down" using a recently published ontogeny for CYP3A4[10] and adjustments in absorption and distribution parameters for pediatric subjects < 12 years of age. The model was qualified to predict everolimus PK in pediatric patients with TSC (aged 1 to < 18 years) from EXIST-1 and EXIST-3 (**Supplementary Material S1**).
- Although also a substrate for P-gp, everolimus has high passive permeability measured *in vitro* and is cleared primarily by metabolism in human (see **Supplementary Material S1** for more details). Intestinal mRNA data suggest stable P-gp expression from the neonate up to the adult;[14-17] therefore, no impact of age (≥6 months of age) on everolimus absorption relating to intestinal P-gp is expected. P-gp expression in the kidney and liver appear to mature with age.[18,19] Although we cannot rule out that there may be differences in tissue distribution with age, everolimus is mainly systemically cleared in human via metabolism by CYP3A4 and not by P-gp. Exposure was then simulated for patients who started everolimus at an age older than 6 months and used as a regressor by the following models to predict the short-term and long-term responses.

Prediction of efficacy

Predictions for short-term efficacy were made using a population PKPD (PopPD) count data model for everolimus in TSC-associated POS indication that was described in our earlier publication.[20]

Predictions for the long-term efficacy were made using a linear mixed effect exposure–response model built and qualified on the long-term PD data from EXIST-3 using time-normalized predicted exposure (TNC_{min}). The model used simulated concentrations from the PBPK model for the 200 subjects. It consisted of a multiplicative linear regression model, where TNC_{min} was computed from the start of everolimus up to 96 weeks of treatment for every 12-week interval. The parameter estimates from this model fitted on the EXIST-3 data first were used to predict postbaseline TSC-associated refractory seizure frequency ($SF_{i,j}$) in the simulated patient population for 10 scenarios, where the patients started treatment at the age of 6 months to 2 years and were treated for a period of 2 years (96 weeks). The exposure–efficacy relationship was independent of age. The model considered log-transformed SF at baseline ($SFBL_i$), the number of days the patient was on everolimus treatment (t_j), and the log-transformed TNC_{min} ($p_{TNCmin\ i,j}$) as covariates, and patient as the random effect. A random subject effect on the slope of days on everolimus treatment was also included. An unstructured covariance structure was assumed for the random effects. The model equation is expressed as follows for subject i at each 12-week time window j:

$$\log(SFi,j) = \mu + s_i + \beta_1 \times \log\left(SFBL_i\right) + \beta_2 \times \log\left(p_{TNC\,min\,i,j}\right) + \left(\beta_3 + s_i'\right) \times t_j + \varepsilon_{i,j}$$

where $p_{TNCmin\ i,j}$ is the simulated TNC_{min} from observed data for model building and predicted by the PBPK model for predictions in the target population, and s_i and s_i' are the random effects for patient i.

$$SF_{i,j} = 7 \times \frac{NST_{i,j}}{DDT_{i,j}}$$

NST is the number of TSC-associated refractory seizures recorded during the j^{th} time window, and DDT is the number of nonmissing seizure diary days in the j^{th} time window.

Note that it was observed that the time effect based on EXIST-3 was very small (the slope associated to covariate "time" β_3 was close to zero), indicating that the effect of time in the exposure–efficacy relationship indicating a disease-modified effect was minor. Because the estimated slope for the effect of time was negative, having this "time" effect in the model induced a small continuous reduction in SF.

Assumptions and limitations

For the correct interpretation of this analysis, the following assumptions and limitations were considered:

- The predicted exposure from the PBPK model assumes similar dosing for all patients and no potential dose titrations, which might occur in the clinical settings.
- The exposure–efficacy analysis based on the EXIST-3 data did not identify the use of CYP3A4 inducer/inhibitors as statistically significant factors influencing the exposure–efficacy relationship, and therefore these factors were not considered in the statistical models.
- Patients who entered the EXIST-3 study were supposed to have a stable regimen of antiepileptic drugs (AED) at baseline and during the core phase of the EXIST-3 study. The AED regimen was not controlled during the extension phase. Potential changes in concomitant AED regimen during the extension phase might have had some effect on efficacy. For these predictions, it was assumed that the AED regimen is comparable with what it was in the EXIST-3 study.
- The long-term exposure–efficacy statistical model is exploratory in nature and was used to utilize the long-term efficacy data available in the EXIST-3 study (up to 2 years). A qualification of this model via external data was not provided. Model goodness of fit and diagnostics plots are described and provided in **Supplementary Material S1**.
- The long-term prediction of efficacy via a linear mixed model might induce an unrealistic continuous declining effect in the long term.

RESULTS

PK simulations

The final PBPK pediatric model predicted the mean body surface area–corrected blood clearance per os (CL_{po}) within ~10% of the actual value for ages 1–12 years. For children aged 12–18 years, the simulations were within ~30% of the actual value (**Table S1**). The dose-normalized PK parameters (C_{min}, area under the concentration-time curve during 24 hours, and peak plasma concentration) for children, measured within a substudy of EXIST-3, were also well predicted by the PBPK models (e.g., age 3 to < 10 years within 15%; **Table S2**).

The ultimate goal of the PBPK model development was to predict the exposure of everolimus in infants from 6 months of age to 1 year. Initial simulations indicated that redefining age with the time of simulation was important to capture in the model. As shown in **Figure 2**, the change in the demographics and physiology between a 6 month old and 1.5 year old was apparent in the change in clearance during the 1-year period of dosing. Therefore, final simulations to apparent steady state in pediatric subjects (up to 2 months of dosing) were done using the "redefine subjects over time" feature in Simcyp. A summary of the final predictions of median steady-state PK parameters, C_{min}, and area under the concentration-time curve during 24 hours is shown in **Figure 3**, and the values are presented in **Table S3** along with the administered dose in milligrams, median body surface area of the simulated population at the start and end of the simulation, and the resultant dose in mg/m^2. The target dose was 6 mg/m^2 for these age groups of patients starting everolimus at an age between 6 months to 2 years. Actual doses ranged from 2–4 mg.

Prediction of short-term efficacy of everolimus in pediatric patients with TSC

The short-term efficacy of everolimus among pediatric patients in reducing the %RSF and responder rate at the end of 24 weeks of treatment from baseline was predicted using the PBPK-predicted C_{min} at the end of 24 weeks of treatment (7.7–10.5 ng/mL, well within the targeted range of 5–15 ng/mL).

Qualification

For patients aged between 6 and 56 years, the PopPD model predicted similar %RSF values to those predicted at C_{min} values of 5, 10, and 15 ng/mL for the EXIST-3 population, with %RSF values between 32.74% and 52.69% (**Table 1**). This is similar to the reduction in %RSF that was reported in the primary analysis of EXIST-3 for the high-target group (40.0%),[6] which included a large proportion of patients older than the age of 6 years (71.5%) who received everolimus treatment for a similar duration (median 18 weeks; range 3–21 weeks). The minor differences are likely the result of the differences in populations where PBPK predictions were made for patients of a predetermined age and dose, and the EXIST-3 population predictions followed the patients' ages (shown stratified by age groups) and dosing regimen.

Predictions

The model predicted a substantial RSF among pediatric patients at the predicted C_{min}. The median predicted %RSF was at least 64.2% (5th quantile; 95th quantile: 46.1%; 74.9%) at 24 weeks from the start of everolimus treatment. The predicted highest %RSF was 77.8% (5th quantile; 95th quantile: 60.6%; 87.6%) at 24 weeks from the start of everolimus treatment (**Figure 4**, **Table S4**). These results were in line with the influence of age on the onset of effect, as identified in the PopPD model reported previously.[20] The predicted responder rate at the 24-week C_{min} was at least 57.5% (5th quantile; 95th quantile: 52%; 64%) for patients who started everolimus at 22 months. The predicted highest responder rate was 67.5% (5th quantile; 95th quantile: 61.5%; 72.5%) at 24 weeks.

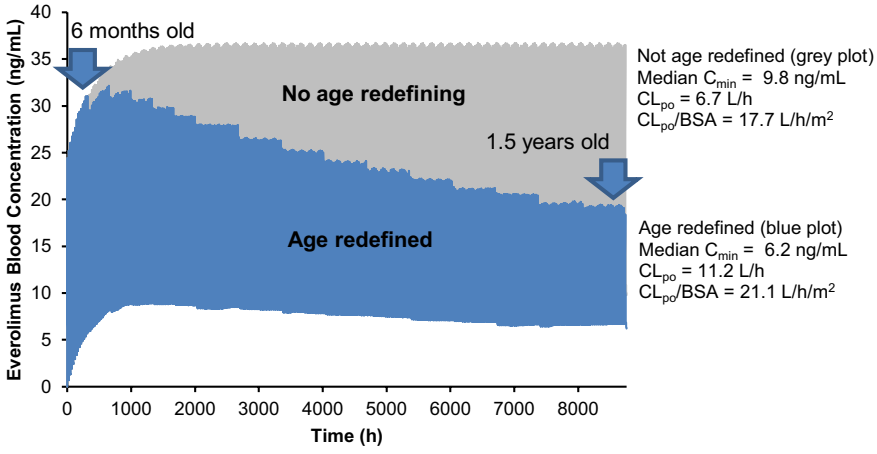

Figure 2 Physiologically-based pharmacokinetic model prediction of everolimus concentrations in a 6 month old during a 1-year period. In these simulations, 6 month olds (n = 100) were treated with 2 mg once daily for 1 year either age-redefining the population with time or not. The blue simulated concentration-time profile depicts simulations where demographics and physiology are changing with time (age-redefining with time, i.e., patient is 1.5 years old at the end of the simulation). The gray profile depicts no age refinement with time (i.e., the infant is still 6 months old at the end of the simulation). A difference in exposure (C_{min}) and clearance at the end of the simulation is apparent for the two modeling scenarios. BSA, body surface area; CL_{po}, clearance per os; C_{min}, trough plasma concentration.

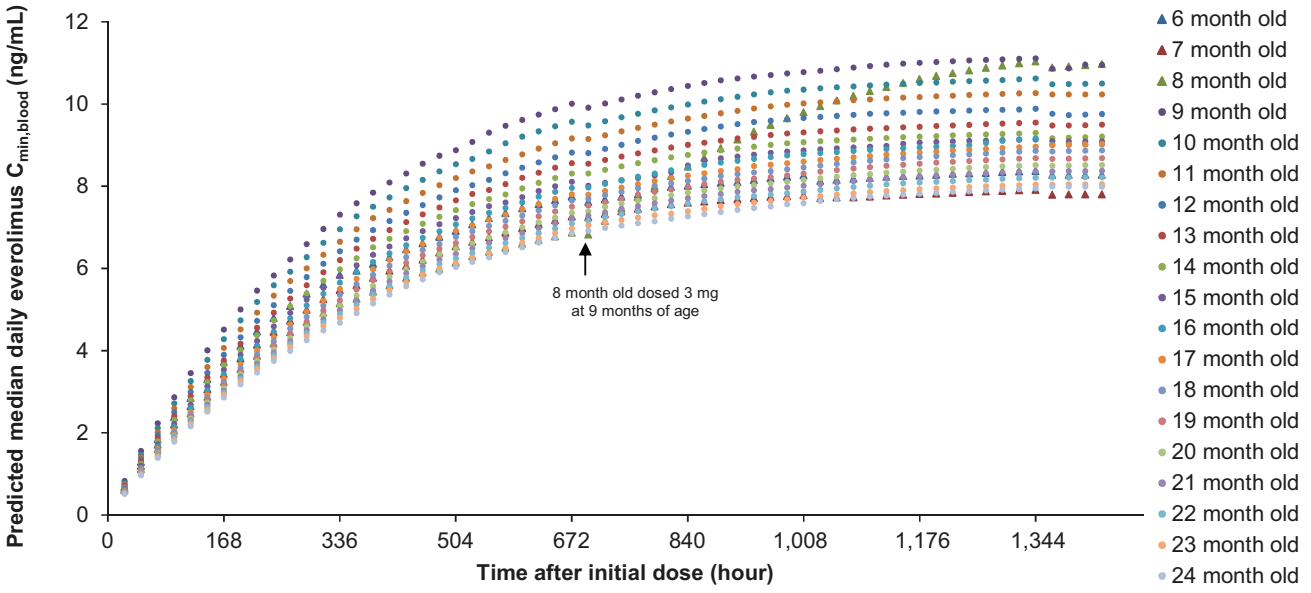

Figure 3 Physiologically-based pharmacokinetic model predicted median daily everolimus blood trough concentration ($C_{min,blood}$) values during a 2-month period in pediatric patients with tuberous sclerosis complex aged 6–24 months at the initial dose in the absence of cytochrome P450 3A4/phosphoglycoprotein inducers. The triangle and circle symbols represent, respectively, 2 and 3 mg doses. Of note, the patients starting everolimus at 8 months had their dose increased to 3 mg at 9 months (green triangles).

Table 1 PopPD model qualification: M&S simulated study and EXIST-3 predicted response after 6 months of treatment

Population	Age at start of everolimus, y	Median C_{min} (ng/mL)	Predicted median RSF, %
M&S simulated patients	6	6.29	50.49
	6	7.86	52.69
	12	5.95	43.79
	18	5.49	40.54
	18	9.88	43.45
	29	6.09	37.18
	29	9.13	38.74
	56	6.84	32.74
	56	12.31	35.64
EXIST-3 patients	> 18	5	38.46
	> 6 and < 18	5	36.84
	> 18	10	41.46
	> 6 and < 18	10	41.51
	> 18	15	46.15
	> 6 and < 18	15	44.78

C_{min}, trough plasma concentration; EXIST-3, NCT01713946; M&S, modeling and simulation; PopPD, population pharmacodynamics; RSF, reduction in seizure frequency.

Prediction of long-term efficacy of everolimus in pediatric patients with TSC

The long-term efficacy of everolimus among pediatric patients in RSF during 96 weeks of treatment from baseline was predicted using the linear mixed effect model described in the Methods section. Model estimates are provided in **Supplementary Material S1**. To evaluate the model fit of the two models based on the EXIST-3 data, standard diagnostic plots were generated. Graphical comparison showed a good agreement between the observed values and the predicted values, indicating no major deviations from the model assumptions. Visual inspection of the studentized residuals did not reveal any clear patterns (**Figure S1**).

Based on the analysis of the EXIST-3 study data, a 0.5-fold reduction in baseline SF was predicted to reduce SF by 49.41% (95% confidence interval (CI), 45.68–52.89). A two-fold increase in TNC_{min} within the exposure ranges observed would result in an additional 21.39% average reduction in postbaseline SF (95% CI, 13.30–28.74). Every additional 12 weeks of exposure to everolimus was predicted to have a modest but significant effect on SF, resulting in a decrease of 5.64% (95% CI, 3.54–7.70). These parameter estimates were used to generate the long-term efficacy predictions.

As the parameter estimate for the log-transformed baseline TSC-associated refractory SF was very close to one (0.9832), the derived percentage reduction from baseline was not greatly affected by the baseline value used in the predictions. Consequently, TNC_{min} and the time on treatment drove the long-term efficacy predictions of the percentage reduction from baseline.

Overall, a pronounced decrease in percentage frequency from baseline was noted across all 10 everolimus starting-age scenarios. Because of the low time-normalized C_{min} and the short period of treatment, there were a few outliers at the first few time points. However, over time, because of the time effect and steady TNC_{min} values, the model predicted a continued reduction from baseline in SF with time on therapy (**Figure 5, Table S5**). On average, a 50% reduction or more from baseline was achieved at the first 12-week time point and a reduction of around 70% at the final time point (96 weeks).

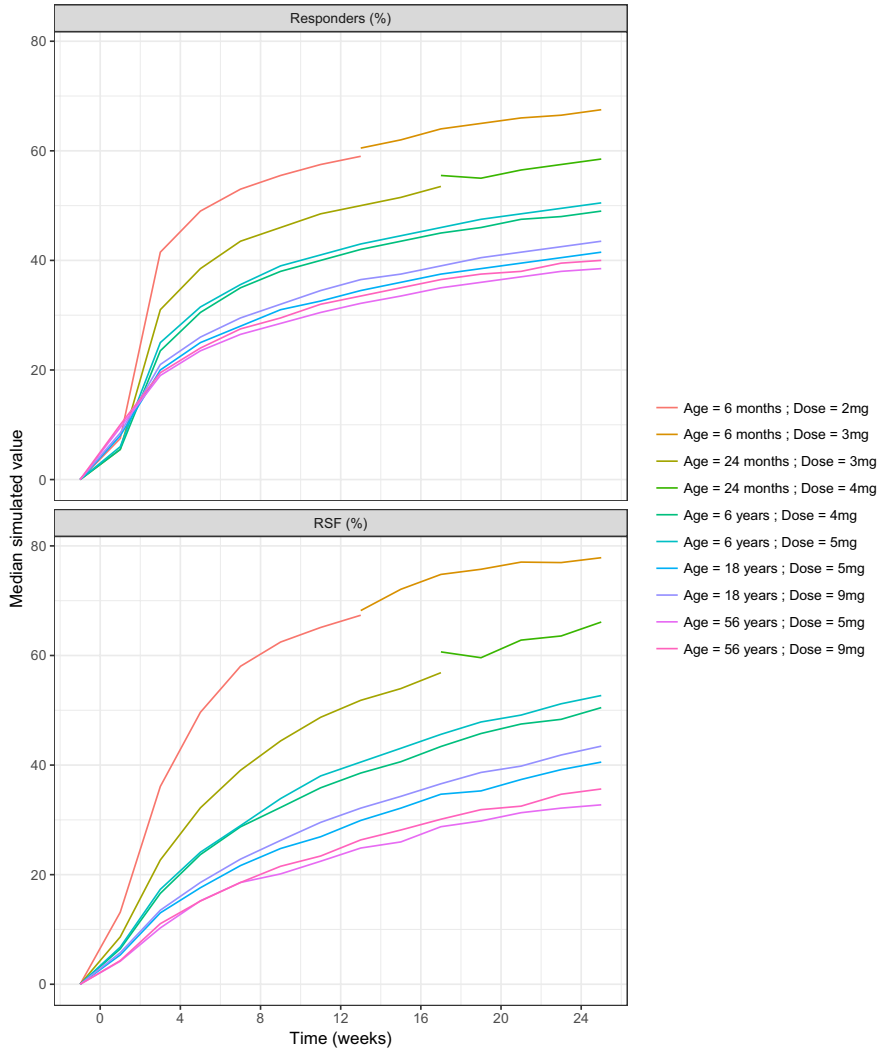

Figure 4 Predicted short-term efficacy of everolimus. RSF, reduction in seizure frequency.

DISCUSSION

The short-term and long-term efficacy of everolimus in TSC-associated POS in patients in the age group of 6 months to 2 years was predicted by a model-based exposure–response analysis. Three models—the PBPK-based, PopPD, and linear mixed effect regression models—were employed in tandem to perform the predictions. A PBPK-based model was used to predict everolimus PK after single and multiple doses as PBPK modeling has been recommended for young children particularly children particularly those aged younger than 2 years.[21] The PBPK model accounts for demography, physiology, and ontogeny of CYP3A4.[22] The predicted PK concentrations in patients with TSC who started everolimus between the ages of 6 months and 2 years were used to predict the short-term and long-term efficacy of everolimus in SF reduction. EXIST-3 was the source of efficacy data. Assumptions in the original models were not changed for the purpose of the predictions in this report.

In our bridging study, we used both a mechanistic PK model and an empirical PD model to bridge the prediction of efficacy in a target population where recruitment would be impossible. PBPK model for pediatric extrapolation is well known and accepted. The complexity of the disease and a lack of knowledge of the mechanism of action would prevent the development of a more mechanistic PD model. A bridging study using an empirical PD model such as presented in this article is the only means for providing information in a population where recruiting in a traditional study (such as EXIST-3) would be challenging. Confidence in the PD model in being able to extrapolate to slightly younger ages is increased by the large number of patients similar to the target population. Indeed, about 80% of patients recruited in the EXIST-3 study were younger than 18 years old, including 28 patients younger than 3 years old and 104 younger than 6 years old.

The EXIST-3 study results recommended a starting dose of 6 mg/m^2 for TSC-associated POS in patients < 6 years of age. The same dose was used in the simulations for this study.

The predicted everolimus trough concentrations at a dose of 6 mg/m^2 in the age group of 6 months to 1 year were within the target range of 5–15 ng/mL on average and consistent

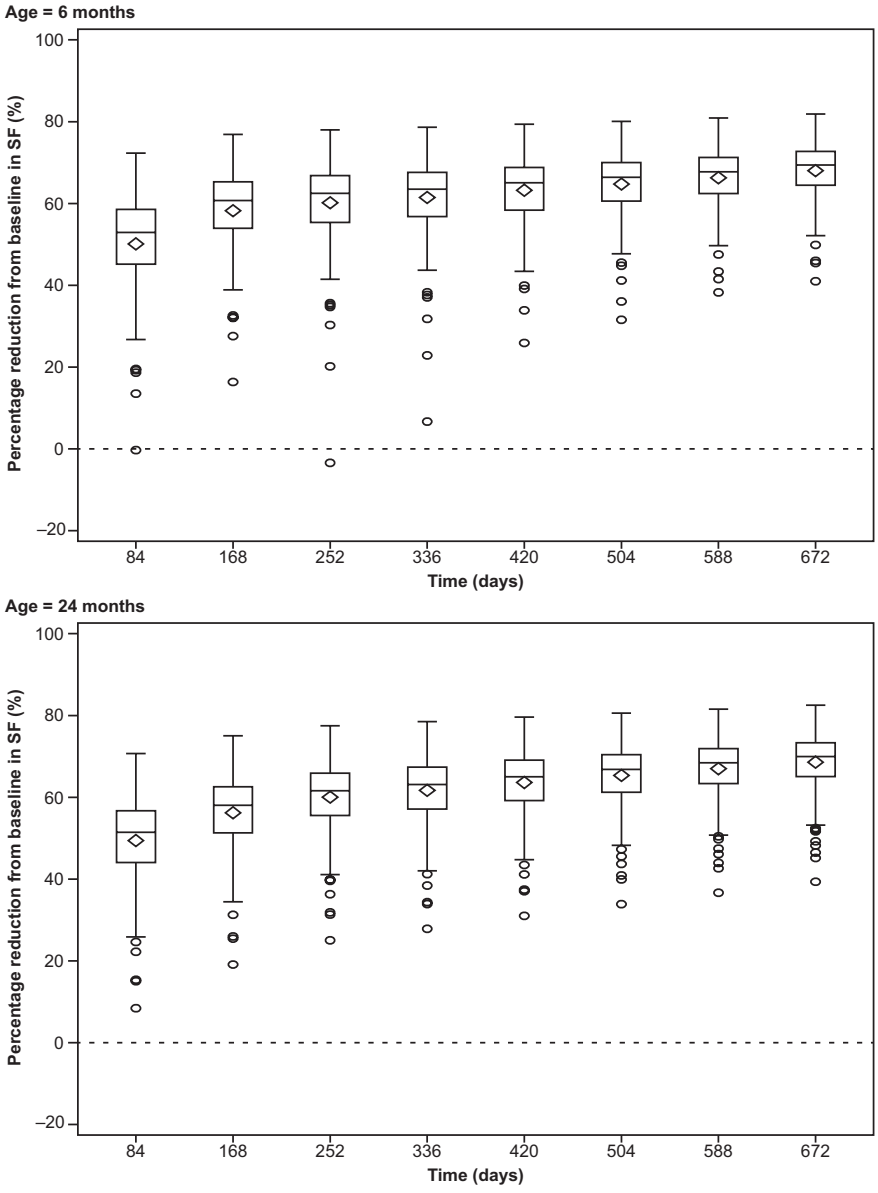

Figure 5 Boxplots of predicted percentage reduction from baseline in SF during 12-week intervals by baseline SF and by age at start of everolimus. SF, seizure frequency.

with the observed concentrations in patients older than 1 year of age. The predicted concentrations in the age group of 6 months to 1 year were slightly higher than those observed in adults (similar to the high-target cohort in EXIST-3), as the PBPK model did not take into account individual dose changes as a result of therapeutic drug monitoring.

Exposure–efficacy analyses predicted that TSC patients aged between 6 months and 2 years would experience a reduction in TSC-associated refractory SF at both short-term and long-term exposures. In fact, this effect could be observed as early as 12 weeks into therapy when patients have reached the steady state. The predicted median RSF was in the range of 40–70%, more noticeable among younger patients. This was higher than the reduction in baseline SF (30–40%) observed in adult patients in the EXIST-3 study, which can be expected given the higher steady-state C_{min} observed in patients 6 months to 2 years of age and the age effect included in the everolimus PD model. In addition, the PopPD models included an age effect, leading to a quicker response in children. From the long-term efficacy analysis based on the EXIST-3 data, seizure control was assumed to improve with continued therapy with everolimus, and thus, an increased RSF was predicted with continued exposure for up to 2 years (96 weeks).

It is important to note that the exposure–response models assume similar exposure–response relationships between adults and children. The predictions made by short-term efficacy models for adults were similar to that observed in EXIST-3. The predictions for children may be considered to be valid and robust.

The main PD end point used in that study is the epilepsy count, which place the site of action likely in the brain, whereas the exact mechanism is not fully elucidated. One hypothesis to explain the reduction of seizures with everolimus effect states that the reduction of subependymal giant cell astrocytoma lesions in the brain lead to a lower seizure count.[23,24] Given the lack of exact knowledge on the mechanism, a mechanistic model may be difficult to establish. Therefore, a published PK/PD count data Poisson model that would address the potential delay between exposure (C_{min}) and effect by its effect on the hazard rate was seemed appropriate to predict efficacy in patients older than 6 months of age. Several results of the population PKPD model as related in Combes *et al*.[20] find a justification in clinical practice. For example, because children's brains are more sensitive than the brains of adults, the baseline seizure count is likely to be higher. This relationship has been found in the population PKPD model as well along with a higher everolimus effect on the Poisson distribution parameter lambda. To the author's knowledge, no further difference in disease and everolimus effect between a 6-month-old and a 2-year-old patient was observed nor is expected. Of note, about 28 patients younger than 3 years old (104 younger than 6 years old) were recruited in the study: The model was developed and validated based on a population of both pediatric and adult patients.

Based on modeling, everolimus at 6 mg/m^2 dose is expected to be efficacious in reducing daily SF associated with TSC-associated POS in patients 6 months to 2 years of age in both the short term (i.e., 12 weeks of exposure) and long term (i.e., up to 2 years of exposure).

Supporting Information. Supplementary information accompanies this paper on the *CPT: Pharmacometrics & Systems Pharmacology* website (www.psp-journal.com).

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Conflict of Interest. All authors are or were employees of Novartis at the time of the research. None of the authors declares any conflict of interest.

Author Contributions. All authors wrote the manuscript. All authors designed the research. F.P.C. and H.J.E. performed the research. F.P.C. and H.J.E. analyzed the data.

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