A Prospective Population Pharmacokinetic Analysis of Sapropterin Dihydrochloride in Infants and Young Children with Phenylketonuria

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Published online: 23 October 2014
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Abstract

Background and Objectives Untreated phenylketonuria (PKU), a hereditary metabolic disorder caused by a genetic mutation in phenylalanine hydroxylase (PAH), is characterized by elevated blood phenylalanine (Phe) and severe neurologic disease. Sapropterin dihydrochloride, a synthetic preparation of naturally occurring PAH cofactor tetrahydrobiopterin (BH4), activates residual PAH in a subset of patients, resulting in decreased blood Phe and increased Phe tolerance. The objective of this study was to determine the appropriate dose of sapropterin in pediatric patients (0–6 years). The study design used D-optimization and was prospectively powered to achieve precise estimates of clearance and volume of distribution.

Methods Oral sapropterin (5 or 20 mg/kg) was administered once daily. Sapropterin plasma concentrations were measured by a validated method. Population pharmacokinetic analysis was performed with NONMEM version 7.2 on pooled data from 156 pediatric and adult PKU patients in two phase III clinical studies.

Results The best pharmacokinetic model was a one-compartment model with an absorption lag, first-order input, and linear elimination, with a factor describing endogenous BH4 levels. Body weight was the only covariate significantly affecting sapropterin pharmacokinetics. Based on recommended dosing, exposure across age groups was comparable. The absorption rate and terminal half-life suggest flip-flop pharmacokinetic behavior where absorption is rate limiting.

Conclusion The effect of weight on sapropterin pharmacokinetics was significant and exposure was comparable across age groups; thus, weight-based dosing is appropriate. The doses selected for pediatric patients provided similar exposure as in adults. Given the slow absorption and elimination half-life, once-daily dosing is justified.

Key Points

Population pharmacokinetics of sapropterin dihydrochloride, a synthetic preparation of naturally occurring phenylalanine hydroxylase cofactor tetrahydrobiopterin (BH4), were evaluated in pediatric phenylketonuria patients. The best pharmacokinetic model was a one-compartment model with an absorption lag, first-order input, and linear elimination, with a factor describing endogenous BH4 levels. Body weight was the only covariate significantly affecting sapropterin pharmacokinetics. The doses selected for pediatric patients provided similar exposure as in adults.

1 Introduction

Phenylketonuria (PKU) is a hereditary metabolic disorder caused by a genetic mutation and deficiency in...
phenylalanine hydroxylase (PAH), an enzyme required for the metabolism of phenylalanine (Phe). In PKU patients, PAH is mutated to varying degrees and if active PAH is not present in sufficient quantities, Phe accumulates to abnormally high levels in the blood and brain; this often results in mental retardation and brain damage, mental illness, seizures, tremors, and cognitive problems. Tetrahydrobiopterin (BH4), a cofactor for PAH, facilitates the hydroxylation of Phe to tyrosine, thereby maintaining appropriate levels of plasma Phe. Several studies have shown a reduction in plasma Phe levels in some PKU patients treated with a synthetic preparation of the dihydrochloride salt of naturally occurring BH4 (sapropterin dihydrochloride; sapropterin) [1–3].

A population pharmacokinetic model of data arising from study PKU-004, which assessed sapropterin levels in children (≥9 years of age) and adults, found a two-compartment model with endogenous BH4 provided the best description of the data [4]. The population pharmacokinetic model from this study was used to design an optimal pharmacokinetic sampling strategy for study PKU-015, a population pharmacokinetic study to characterize pharmacokinetic characteristics of BH4 in pediatric patients from 0 to 6 years of age, and provide dosing recommendations for this population. The study design used D-optimization [5–7] based on the previous pharmacokinetic model and was prospectively powered to achieve precise estimates of CL/F and Vc/F in each age group.

Graphical examination of the concentration–time data arising from PKU-015 suggested that there were little data supporting a peripheral compartment, which was reflected in the poor precision of the estimates for the associated parameters in the previous evaluation (Electronic Supplementary Material Fig. 1S). Thus, pooled data from studies PKU-004 and PKU-015 were fit to ensure a pharmacokinetic model that was capable of describing data arising from all ages.

2 Methods

2.1 Study Design

Both studies (PKU-015 and PKU-004) were approved by institutional review boards or ethics committees at all centers and were performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. Informed written consent was obtained from all patients or their guardian, in the case of children, before inclusion in the study.

2.1.1 Study PKU-015

Study PKU-015 was an open-label, multicenter study conducted at 19 centers in the USA and Canada. The study was designed to evaluate the safety and population pharmacokinetics of sapropterin, and the effect of sapropterin on neurocognitive development and blood Phe levels in infants and young children with PKU. A total of 95 patients (0–6 years old) received oral sapropterin at a dose of 20 mg/kg once daily (at the same time each day) with food for 4 weeks. Subjects were eligible to participate in the population pharmacokinetic substudy and were enrolled by age at study entry. A total of 94 subjects were enrolled in the pharmacokinetic substudy and 80 of these subjects were evaluable. D-optimization was used to select the pharmacokinetic sample times and identify the number of patients needed to estimate the pharmacokinetic parameters. The study was prospectively powered to achieve precise estimates of CL/F and Vc/F in each age group.

2.1.2 Study PKU-004

Study PKU-004 was a multicenter, intra-patient, dose-escalation, open-label extension study conducted at 26 centers in North America (Canada and the USA) and Europe (France, Germany, Ireland, Italy, Poland, and the UK). The study was designed to evaluate the long-term safety and efficacy of various doses of sapropterin in patients ≥8 years old with PKU who had previously responded to sapropterin treatment. Study PKU-004 occurred in two parts. In part 1, patients received sapropterin in three consecutive 2-week courses of daily single oral doses of 5 mg/kg, followed by 20 mg/kg/day, and finally 10 mg/kg/day for 4 more weeks. Following completion of the 4-week 10 mg/kg/day period in part 1 of PKU-004, each patient was enrolled in part 2, a 16-week fixed-dose period during which the daily dose of sapropterin was fixed within the range of 5–20 mg/kg/day on the basis of the patient’s Phe level at the end of the 2-week 10 mg/kg treatment period. A total of 80 subjects were enrolled in study PKU-004. After completing the first 16 weeks of treatment, 78 subjects were enrolled and evaluable in the pharmacokinetic substudy.

2.2 Pharmacokinetic Sampling

In PKU-015, three plasma samples from each patient in the ≤1 year old age group and four plasma samples from each patient in the >1 year old age group were collected at the week 0 through week 4 visits according to a D-optimized design shown in Table 1.

In PKU-004, four plasma samples from each patient were collected from each patient at any point during the...
week 16, 20, or 22 visits according to a D-optimized design shown in Table 1. Patients receiving 10 mg/kg/day in the fixed-dose period could be assigned to Group 1 or Group 2, but were to follow the prescribed sample assessment schedule for that group once they had been assigned.

2.3 Tetrahydrobiopterin Assay

As BH4 is unstable in plasma, its concentration was measured indirectly by oxidizing BH4 to L-biopterin; measuring the concentration of L-biopterin, which has been shown to be stable [4, 8]; and correcting for the oxidative conversion of BH4 to L-biopterin. The collected plasma samples were shipped frozen on dry ice to a central laboratory for assay. Samples were oxidized and assayed for L-biopterin by a validated analytical liquid chromatography/tandem mass spectrometry method.

The plasma concentration of BH4 was calculated from the measured concentration of L-biopterin by correcting for the oxidation conversion ratio of BH4 to L-biopterin, which was determined in the assay validation as the molar ratio, according to equation (Eq. 1):

\[
\% \text{BH4 conversion ratio} = \frac{\text{Mean of biopterin measured}}{\text{Nominal BH4 concentration}} \times \frac{\text{MW}_{\text{BH4}}}{\text{MW}_{\text{Biopterin}}} \times 100
\]

where \(\text{MW}_{\text{BH4}}\) is 241.2 g/mol and \(\text{MW}_{\text{Biopterin}}\) is 237.2 g/mol. The nominal conversion ratio of BH4 to L-biopterin was determined to be 47.3 %. The conversion ratio was stable within at least 8 weeks of sample storage at \(-70^\circ C\).

The lower limit of quantitation was 5.00 ng/mL for L-biopterin and 10.7 ng/mL for BH4.

2.4 Pharmacokinetic Modeling

The pharmacokinetic data used in the population analysis included all available concentration data collected from studies PKU-015 and PKU-004. Data from study PKU-015 were intended to be evaluated separately from study PKU-004, or if needed using an informative prior based on the previously developed population pharmacokinetic model [4]. However, initial graphical evaluation of data from study PKU-015, together with preliminary model-based evaluations, suggested that the data for both studies were adequately described using a one-compartment model with a parameter for endogenous BH4, rather than a two-compartment model as reported for PKU-004 [4]. Subsequently, the data from studies PKU-015 and PKU-004 were pooled for population pharmacokinetic model development.

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2.4.1 Structural Model and Covariate Model

A series of different structural models were evaluated in the present analysis, including one- and two-compartment models with first-order input, with and without an absorption lag. The best pharmacokinetic model was a one-compartment model with an absorption lag, first-order input, and linear elimination, with a factor describing endogenous BH4 levels. Covariates evaluated in the model development are listed in Electronic Supplementary Material Table 1S and body weight was the only covariate significantly affecting sapropterin pharmacokinetics. The first-order conditional estimation (FOCE) method was used because the change in objective function value (ΔOFV) has been found to more reliably follow a chi-square distribution.

Continuous covariates such as age or weight were modeled using a general power function, according to Eq. 2:

\[ \text{TVP} = P_{\text{pop}} \cdot \prod_{i=1}^{n} \text{cov}_i^{\theta_i} \]  

where the typical value of the parameter (TVP) represents the predicted pharmacokinetic parameter (CL/F, \( V_c/F \)) for the 'reference' individual with covariate value(s) \( \text{cov}_i \), \( P_{\text{pop}} \) represents the population central tendency for the pharmacokinetic parameter TVP, \( \text{cov}_i \) represents the individual value for that covariate normalized by the approximate median value for the patient population, and \( \theta_i \) represents a scale factor relating the covariate function to the pharmacokinetic parameter. A typical adult (e.g., 70 kg body weight) was chosen as the reference individual to allow comparison of the results in pediatrics with those in adults.

Categorical covariates (e.g., race) were modeled using the general Eq. 3:

\[ \text{TVP} = P_{\text{pop}} \cdot \theta_i^{\text{cov}_i} \]  

where \( \text{cov}_i \) is either 0 for the standard or reference patient, or 1 for the comparative patient. \( P_{\text{pop}} \) represents the value for the pharmacokinetic parameter when \( \text{cov}_i \) is 0 and \( \theta_i \) represents a scale factor for the influence of that covariate on the pharmacokinetic parameter.

Covariates were first examined for potential effect on structural parameters by graphical assessment in Xpose 4.3.5 running on R3.0.2, followed by a model-based analysis if any trend was observed. A standard approach was used in covariate evaluation. During individual covariate evaluations, a \( P \) value of 0.005 was used to select covariates; a full model was built with all identified covariates and a \( P \) value of 0.001 was used to reduce the model during backwards elimination. Covariate factors were considered clinically relevant and accepted in the model if the magnitude of the change of the parameter due to a covariate influence resulted in \( \geq 20\% \) variation of the parameter. The precision of the estimated covariate effect was also considered; covariate factors were not accepted unless the parameter precision was \( < 30\% \).

2.4.2 Statistical Model

Inter-individual variability was described using the following exponential error model, according to Eq. 4:

\[ P_j = \text{TVP} \cdot e^{\eta_j} \]  

where \( P_j \) is the individual value for the pharmacokinetic parameter in the \( j \)th individual and \( \eta_j \) is an independent random variable with a mean of zero and variance \( \omega_p^2 \). Between-occasion variability was also assessed.

The residual variability for the pharmacokinetic model was described using a constant coefficient of variation (CCV) model based on the log transform both sides (LTBS) approach according to Eq. 5:

\[ \ln(\text{Cp}_{ij}) = \ln(\hat{\text{Cp}}_{ij}) + \nu_{ij} \]  

where \( \hat{\text{Cp}}_{ij} \) is the \( i \)th plasma concentration for the \( j \)th individual predicted by the model, \( \text{Cp}_{ij} \) is the measured concentration, and \( \nu_{ij} \) is the residual error normally distributed with mean zero and variance \( \sigma^2 \).

2.4.3 Model Selection Criteria

Model building followed standard criteria [9, 10]. Decision making during model building was guided by evaluation of the ΔOFV, evaluation of the magnitude of inter-individual and residual variance, and examination of diagnostic residual plots. The chi-squared test \( (P < 0.005) \) for the log-likelihood ΔOFV between nested models with degrees of freedom equal to the difference in number of parameters between models was used to declare superiority of one model over another. This corresponds to a reduction in OFV of \( \geq 7.88 \) \( (P < 0.005) \) for comparison of models that differ by one parameter. The superior model should also have an associated reduction in the magnitude of inter-individual and residual variance estimates, as well as improved diagnostic plots. The ΔOFV was not used to evaluate parameters describing variability. The covariance step was implemented for each model, and standard errors (SEs) for parameter estimates, as well as correlation between parameters, were evaluated. Models with parameter estimates with high associated SEs (>35 % of the parameter estimate), models with a high degree of correlation between parameters (>90 %), and models that included a covariate(s) whose effect on the estimated parameter value was negligible were carefully evaluated and re-parameterized, or possibly rejected.
2.4.4 Model Qualification

The model developed in this analysis was tested and qualified by evaluating precision of the final parameter estimates and the condition number, and determining both the symmetrical 95% confidence intervals (CIs) from the asymptotic SEs of the parameter estimates, as well as non-parametrically bootstrapped 95% CI. Standard diagnostic plots and visual and numerical predictive checks were also evaluated. Finally, the power to evaluate CL/F and Vc/F was evaluated using the final model and database to confirm the prospective evaluation.

In addition, a visual predictive check (VPC) [11] was conducted to compare the distribution of simulated observations from the final model to those obtained from the original data.

2.5 Systems

The concentration–time data collected in these studies were analyzed using mixed-effects modeling methods as implemented by the computer program NONMEM® (version 7, level 2; Icon Development Solutions, Ellicott City, MD, USA) [12, 13], compiled using Intel Fortran Parallel Studio 2011 (Intel Corporation, Santa Clara, CA, USA), and installed on a grid server system running Windows Server 2008 x64-bit. Diagnostic graphics, exploratory analyses, and post-processing of NONMEM® output was performed using the SPlus 6.2 Professional software [14] or R version 2.15.0 or later.

3 Results

3.1 Demographics

A total of 156 patients (80 females, 76 males) took part in this study. Age groups were <1 year (n = 10), 1 year to <2 years (n = 14), 2 years to <4 years (n = 28), 4 years to <7 years (n = 28), 7 years to <12 years (n = 10), and ≥12 years (n = 66). The overall summary statistics for baseline demographics are presented in Table 2.

| Table 2 Demographic characteristics of study patients |
|------------------------------------------------------|
| Characteristic                                      | Study PKU-015 (N = 80)a | Study PKU-004 (N = 78)a | Pooled studies PKU-015 and PKU-004 (N = 156)b |
| Age (years)                                         | 3.28 (2.01) [0.107–6.98] | 21.1 (9.64) [9–50]      | 12 (11.3) [0.107–50] |
| Age category [n (%)]                                |                         |                         |                       |
| <1 year                                             | 10 (12.5)               | 0                       | 10 (6.4)              |
| 1 to <2 years                                       | 14 (17.5)               | 0                       | 14 (9.0)              |
| 2 to <4 years                                       | 28 (35.0)               | 0                       | 28 (17.9)             |
| 4 to <7 years                                       | 28 (35.0)               | 0                       | 28 (17.9)             |
| 7 to <12 years                                      | 0                       | 10                      | 10 (6.4)              |
| ≥12 years                                           | 0                       | 68                      | 66 (42.3)             |
| Sex [n (%)]                                         |                         |                         |                       |
| Females                                             | 49 (61.3)               | 33 (42.3)               | 80 (51.3)             |
| Males                                               | 31 (38.7)               | 45 (57.7)               | 76 (48.7)             |
| Ethnicity [n (%)]                                   |                         |                         |                       |
| Non-Hispanic                                        | 78 (97.5)               | 76                      | 152 (97.4)            |
| Hispanic                                            | 2 (2.5)                 | 2                       | 4 (2.6)               |
| Body weight (kg)                                    | 15.9 (6.36) [4.5–41.9]  | 67.2 (21.8) [28.2–144]  | 40.9 (30.3) [4.5–144] |
| Height (cm)                                         | 95.6 (18.3) [56–128]    | 165 (13.3) [126–191]    | 129 (38.2) [56–191]   |
| Body surface area (m²)                              | 0.635 (0.19) [0.259–1.18] | 1.72 (0.31) [1.05–2.65] | 1.17 (0.604) [0.259–2.65] |
| ALT (IU/L)                                          | 22.4 (8.15) [13–63]     | 28.4 (18.3) [11–127]    | 25.4 (14.5) [11–127]  |
| AST (IU/L)                                          | 38.1 (8.75) [23–63]     | 25.7 (5.8) [14–43]      | 32.2 (9.65) [14–63]   |
| Bilirubin (mg/dL)                                    | 7.51 (4.4) [1.71–34.2]  | 0.55 (0.33) [0.1–1.9]   | 4.12 (4.7) [0.1–34.2] |
| Albumin (g/dL)                                      | 4.19 (0.274) [3.6–4.9]  | 4.49 (0.23) [4–5]       | 4.33 (0.29) [3.6–5]   |
| CLCR (mL/min)b                                      | 57.1 (22.2) [9.39–120]  | 114 (26) [48–231]       | 87.8 (45.1) [9.39–276] |
| Baseline phenylalanine (µmol/L)                     | 324 (140) [57.5–768]    | 811 (393) [53–2190]     | 562 (378) [53–2190]   |

ALT alanine aminotransferase, AST aspartate aminotransferase, CLCR creatinine clearance

a Values are presented as mean (standard deviation) [range], unless specified otherwise

b Calculated from plasma creatinine

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Table 3  Estimated population pharmacokinetic parameters and bootstrap 95 % confidence interval for sapropterin dihydrochloride

| Parameter (units) | Parameter | Final model estimate (SE) | Bootstrap model estimate (95 % CI) |
|-------------------|-----------|---------------------------|----------------------------------|
| CL/F (L/h)        | \( \theta_1 \) | 2.710 (9.8)               | 2.708 (2.308–3.162)              |
| Effect of weight on CL/F | \( \theta_6 \) | 0.864 (7.3)               | 0.861 (0.751–0.980)              |
| \( V_c/F \) (L)  | \( \theta_2 \) | 3.010 (43.9)              | 3.967 (1.509–8.596)              |
| Effect of weight on \( V_c/F \) | \( \theta_7 \) | 0.644 (18.9)              | 0.658 (0.399–0.827)              |
| \( k_{a} \) h\(^{-1}\) | \( \theta_3 \) | 0.235 (23.8)              | 0.284 (0.158–0.524)              |
| \( t_{lag} \) (h) | \( \theta_4 \) | 0.321 (11.2)              | 0.313 (0.246–0.400)              |
| C0 (\( \mu g/\)L) | \( \theta_5 \) | 16.6 (4.1)                | 16.39 (15.5–18.22)               |
| Residual error study PKU-004 (% CV) | \( \eta_1 \) | 21.1 (9.2)                | 20.47 (17.95–26.0)               |
| Residual error study PKU-015 (% CV) | \( \eta_9 \) | 30.2 (12)                 | 30.312 (23.5–37.86)              |
| \( \text{IIV}_{CL/F} \) (% CV) | \( \eta_1 \) | 45.61 (23.3)              | 45.85 (35.05–56.07)              |
| \( \text{IIV}_{V_c/F} \) (% CV) | \( \eta_2 \) | 56.57 (39.4)              | 54.67 (32.14–78.56)              |
| \( \text{IIV}_{C0} \) (% CV) | \( \eta_3 \) | 36.47 (30)                | 36.16 (26.54–48.17)              |
| Corr (CL/F, \( V_c/F \)) | 0.469 (NE) | 0.34 (0.20–0.50)          |                                  |

C0 endogenous levels, CI confidence interval, CL/F apparent total clearance of the drug from plasma after oral administration, Corr correlation between parameters, CV coefficient of variation, IIV inter-individual variability, \( k_{a} \) absorption rate constant, NE not estimated, SE standard error, \( t_{lag} \) lag time, \( V_c/F \) apparent volume of distribution of the central compartment

3.2 Final Model

The final dataset consisted of 475 observations from 156 patients from studies PKU-015 and PKU-004. The final pharmacokinetic model was a one-compartment model with first-order input and elimination. The model was parameterized in terms of CL/F, \( V_c/F \), first-order absorption rate constant (\( k_{a} \)), an absorption lag time (\( t_{lag} \)), and endogenous level of BH4 (C0). The effects of weight on CL/F and \( V_c/F \) were described using a power function. No other covariates were identified as being predictive of pharmacokinetic variability. The residual error model was a CCV model (additive in the log domain) with separate pharmacokinetic variability. The residual error model was parameterized in terms of CL/F, \( V_c/F \), with first-order input and elimination. The model was parameterized in terms of CL/F, \( V_c/F \), first-order absorption rate constant (\( k_{a} \)), an absorption lag time (\( t_{lag} \)), and endogenous level of BH4 (C0). The effects of weight on CL/F and \( V_c/F \) were described using a power function. No other covariates were identified as being predictive of pharmacokinetic variability. The residual error model was a CCV model (additive in the log domain) with separate terms for each study. Inter-individual variability was described for CL/F, \( V_c/F \), and C0 with terms describing the correlation between CL/F and \( V_c/F \). The equations for the final model parameters are provided in Eq. 6 and the parameter estimates for the final covariate model are presented in Table 3.

\[
\begin{align*}
\text{CL/F} &= \theta_1 \cdot \left( \frac{\text{Weight(kg)}}{70} \right)^{\theta_6} \cdot \exp(\eta_1) \\
\text{V_c/F} &= \theta_2 \cdot \left( \frac{\text{Weight(kg)}}{70} \right)^{\theta_6} \cdot \exp(\eta_2) \\
\text{IIV}_{\text{CL/F}} &= \theta_3 \\
\text{IIV}_{\text{V_c/F}} &= \theta_4 \\
\text{Corr (CL/F, V_c/F)} &= \theta_5 \cdot \exp(\eta_3)
\end{align*}
\]

Parameter precision for the final model was generally good, with most parameters being estimated with relative SEs <25 %, with the exception of \( V_c/F \) and the associated inter-individual variability. For the addition of two covariate factors (weight on CL/F and weight on \( V_c/F \)), the objective function decreased 137.26 points. Inter-individual variability on CL/F decreased from 87 to 46 % and on \( V_c/F \) decreased from 78 to 57 %. The residual variability in the final model was acceptable, being 21 % for data arising from study PKU-004 and 30 % from data arising from study PKU-015; this variability was not affected by the addition of weight as a covariate to CL/F and \( V_c/F \). Evaluation of the population mean value of half-life suggests a terminal half-life of 0.78 h, whereas the absorption half-life (calculated as \( \frac{0.693}{k_{a}} \)) is 2.95 h, suggesting flip-flop pharmacokinetic behavior [15], in which absorption becomes the rate-limiting metric of exposure. However, attempts to re-parameterize the model to force absorption to be faster than elimination were not successful. Given the absorption and elimination half-lives, once-daily dosing in pediatric patients is justified.

3.3 Final Model Stability and Predictability

The median values obtained from the non-parametric age-stratified bootstrap evaluation are in good agreement with the original estimated values (Table 3). The CIs for the parameters do not include the null, supporting the inclusion of weight as a covariate for CL/F and \( V_c/F \). In general, the CIs are reassuringly narrow, although the intervals for \( V_c/F \) are wide, as would be inferred from the SEs of the parameter estimates. The condition number was low, suggesting minimal co-linearity; the shrinkage was low for CL/F, but high for \( V_c/F \) in the final model.
3.4 Predictive Performance of the Final Model

Goodness-of-fit plots are provided in Electronic Supplementary Material Fig. 2S and 3S. A plot of observed versus typical predicted BH4 concentration and the plot of observed versus individual predicted BH4 concentration show generally good agreement, with no apparent bias. Despite including weight in the model to describe CL/F and \( \text{VC/F} \), there was still significant unexplained variability in pharmacokinetic model parameters. The plots of conditional weighted residuals versus typical predicted concentrations and conditional weighted residuals versus time after dose show no evident trends or bias, and the range of conditional weighted residuals falls between \( \pm 3 \).

Frequency histogram plots of normalized prediction distribution errors, quantile–quantile plots, and plots of observed concentrations overlaid with typical predicted concentrations versus time after dose suggested that the final model described the observed data properly.

3.5 Visual Predictive Check

To visualize the predictive performance of the final model, several VPC plots were generated. In a plot showing the 2.5th, 50th, and 97.5th prediction intervals for BH4 concentrations overlaid with the observed data (Fig. 1), most of the observed concentrations fall within the prediction intervals, and there are no obvious patterns for observations that fall outside the prediction intervals, which indicates that the model can capture the variability in the observed data. The percentiles of the observed data are consistent with the prediction intervals of the simulated data. The VPC plots stratified by age or weight suggest that the observed and simulated percentiles are in reasonable agreement, even for the lowest age and weight groups in which the amount of data is sparse. A VPC plot stratified by study suggested that, despite simplification of the model originally used to describe data arising from PKU-004, the data are adequately reproduced by the new model, and confirmed that there are no inherent differences between the two study populations.
3.6 Confirmation of Study Power

The desired precision was to target the 95 % CI of the CL/F and Vc/F to be within the intervals of 60 and 166 % of the point estimate for the geometric mean for each designated age group. A prospective evaluation of study power indicated that PKU-015 had sufficient power to meet these criteria. The power was re-evaluated and the results demonstrate that the study had sufficient power to meet the prespecified criteria for each age group (Fig. 2).

3.7 Clinical Significance of Identified Covariates

To assess the clinical significance of the effect of weight, the final population model was used to calculate pharmacokinetic parameter values for patients that represented the extremes of covariate influences within this study population. The effect of weight on CL/F and Vc/F is presented in Electronic Supplementary Material Table 2S. At the extremes of weight, a 5 kg patient had a CL/F and Vc/F value approximately 10 and 18 % of the reference 70 kg patient, respectively. Conversely, a patient weighing 145 kg had CL/F and Vc/F that was 190 and 160 % of the reference patient, respectively. Although the addition of weight to the model significantly decreased the inter-individual variability in CL/F and Vc/F, significant between-patient variability remained.

Both CL/F (Fig. 3a) and Vc/F (Fig. 3b) increase in a non-linear manner with increasing weight, although individual predictions still vary around the typical individual predictions. The intervals for Vc/F are broad, reflecting the precision with which Vc/F was estimated. Although the majority of individual estimated values for Vc/F fall at or below the median, there are patients (particularly patients with low body weight) whose estimated Vc/F values fill the predicted 95 % CI for this parameter.

3.8 Exposure

In simulated concentration–time profiles following 20 mg/kg dosing for a range of weights, concentrations remain above the endogenous level for the dose interval (Electronic Supplementary Material Fig. 4S). Overall, BH4 exposure (area under the concentration–time curve at steady state; AUCss) based on administered dose and individual clearance estimates was comparable across all age groups, although there is a slight visual trend towards higher exposure (AUCss) as the age decreases (Fig. 4). However, the number of patients in the youngest age group is small, so this trend should be considered with caution. Maximum (peak) drug concentration (Cmax) could not be determined as the shrinkage on Vc/F was high and there were no terms for inter-individual variability on kα, making parameters used to calculate Cmax unlikely to reflect individual values. No other covariates were identified.

3.9 Comparison of Previous and Current Pharmacokinetic Models

In order to evaluate the differences in exposure expected from the original two-compartment model and the current one-compartment model, simulated concentration–time profiles for the reference patient were generated. Differences between the expected concentration times arising from these two models is minimal (Fig. 5). The simplification from a two-compartment structural model to a one-compartment structural model used in the current evaluation is justified based further on the poor precision with which the inter-compartmental clearance term (Q/F) and the apparent volume of distribution of the peripheral compartment (Vp/F) were identified in the original model. The inability to utilize the previous model may be partly attributed to the change in NONMEM® versions (6.1–7.2) that involved underlying changes to the FOCE estimation method and changes in the compilers used for NONMEM®.

4 Discussion

This analysis suggests that the pharmacokinetics of BH4 can be described by a one-compartment model first-order input following a tlag and first-order elimination. A term describing the endogenous concentration is also included in the model. Inter-individual variability was included on CL/F, Vc/F, and C0, with correlation described between CL/F and Vc/F. The model was fit using the FOCE method and the LTBS approach was used. A proportional residual error model (additive in the log domain) was incorporated with separate residual error terms estimated for each study.

A D-optimal, sparsely sampled population pharmacokinetic approach was used in this study for several reasons: this approach suggests windows of time where sampling will be most informative relative to a proposed model, without undue penalty against the identification of alternative models; allows patients to have fewer blood samples drawn than with traditional pharmacokinetic sample designs, which often require eight or more pharmacokinetic samples per patient; and weighs various sample schemes based on the efficiency of a proposed design, the expected bias and precision of estimated parameters, and practical considerations. The fact that patients in this study received different dosing regimens of sapropterin further improved the information content of the data obtained. The final model was evaluated using multiple methods including non-parametric bootstrapping and VPCs. All evaluations suggested the model adequately described the data and was acceptable.
Fig. 2 Frequency histograms of individual parameter estimates. Confirmation of power for apparent total clearance of the drug from plasma after oral administration and apparent volume of distribution of the central compartment in the age group 0–1 years (a), 1–2 years (b), 2–4 years (c), and 4–7 years (d). The solid purple line is the median value, dotted black lines are the 95% confidence interval, and the red dashed lines are 60–166% of point estimate of the mean.
Limitations of this study include variability in Phe levels and a small sample size. Phe levels are impacted by multiple factors and can change rapidly in patients with PKU. The timing of Phe determinations relative to pharmacokinetic sampling was not known. Although study PKU-015 was adequately powered and enrollment was consistent...
with other pediatric studies, a relatively small number of pediatric subjects ($n = 80$) are included in this analysis. Total body weight was the only significant covariate and improved the model fit to the data when included on both $CL/F$ and $Vc/F$. The effect of body weight on $CL/F$ and $Vc/F$ was substantial and supports a dose adjustment strategy based on weight in the pediatric population. While the addition of weight to the model significantly decreased the inter-individual variability in $CL/F$ and $Vc/F$, there was still significant between-patient variability remaining. Evaluation of the population mean value of half-life suggests a terminal half-life of 0.78 h, whereas the absorption
half-life (calculated as $0.693/k_a$) is 2.95 h, suggesting flip-flop pharmacokinetic behavior where absorption becomes the rate-limiting metric of exposure. The estimated elimination half-life from the current model is different from the previous model, which could be due to the use of endogenous concentrations to estimate half-life in the previous model.

Based on recommended dosing, exposure across age groups was comparable. Given the absorption half-life and elimination half-life, once-daily dosing is justified in pediatric patients.

5 Conclusion

Overall, the pharmacokinetic model developed in the present evaluation provides reasonable estimates that are consistent with the previous evaluation [4], despite the
simplification of the model. The effect of body weight on CL/F and Vₐ/F was substantial and supports a dose adjustment strategy based on weight in the pediatric population. The doses selected for pediatric patients provided similar exposure. Overall, the exposure across all age groups was comparable and given the absorption and elimination half-lives, once-daily dosing is justified for pediatric patients.

Acknowledgments Analysis of plasma samples for pharmacokinetic analysis was performed by QPS, LLC. (Newark, DE, USA). Database assembly and population pharmacokinetic analysis were conducted by Projections Research Inc. (Phoenixville, PA, USA). Medical writing and editorial assistance were provided by Gary Witherell, PhD (InClin Inc., San Mateo, CA, USA). The authors would like to thank the 19 investigators in study PKU-015 and the additional 24 investigators in study PKU-004 involved in patient recruitment and retention.

Conflicts of interest DRM has provided paid consultancy support to BioMarin Pharmaceutical Inc. YQ, HZ, MM, and DGM are employees of BioMarin Pharmaceutical Inc. and own stock and stock options in BioMarin Pharmaceutical Inc. Projections Research Inc. and InClin, Inc. (San Mateo, CA, USA) were compensated for providing assistance with the study, data analysis, and/or manuscript preparation.

Author contributions YQ, DRM, HZ, MM, and DGM contributed towards the writing and interpretation of the data. The final manuscript was approved by all authors. DRM completed the population pharmacokinetic modeling.

Role of the funding source This study was sponsored by BioMarin Pharmaceutical Inc., which had a significant role in the study design; the collection, analysis and interpretation of data; and the writing of the report. The study protocol was drafted and developed by the study sponsor. Representatives or employees of the sponsor were responsible for the administration and monitoring of the study.

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