CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

22-181

STATISTICAL REVIEW(S)

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research
Office of Translational Sciences
Office of Biostatistics

STATISTICAL REVIEW AND EVALUATION

Clinical Studies

NDA/Serial Number:	NDA 22-181/000
Drug Name:	Sapropterin dihydrochloride (6R-BH4 dihydrochloride, Kuvan, Phenoptin)
Indication(s):	Treatment of phenylketonuria
Applicant:	Biomarin
Date(s):	Received 25 May 2007; PDUFA goal date 25 Nov. 2007
Review Status:	Priority
Biometrics Division:	DB3
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Content and Format

1. EXECUTIVE SUMMARY

1.1. Conclusions and Recommendations

The data support the conclusion that sapropterin is safe and efficacious in lowering blood Phe levels in PKU patients. There is insufficient data, in terms of number and length of studies, to indicate a change in diet for PKU patients receiving sapropterin treatment. For example, there is no information on neurological changes or developmental improvements due to long-term increased dietary phenylalanine. For further discussion, see the medical officer's review.

The design and analysis of the studies are adequate for the indication and use agreed upon in labeling negotiations, namely the lowering of blood Phe in PKU patients. Study PKU-003 provides the primary evidence for this indication, with the flaw that diet in the study patients was not controlled. Study PKU-006, with blood Phe levels designed to be a secondary outcome, provides supportive evidence. Although the difference was not statistically significant, sapropterin seemed to lower blood Phe levels relative to placebo prior to an increase in dietary phenylalanine after Week 3.

1.2. Brief Overview of Clinical Studies

There were two pivotal Phase 3 trials in this application: PKU-003 and PKU-006. In addition, trial PKU-001 served primarily to identify a group of responders for enrollment in PKU-003; the first part (of two) in PKU-006 served a similar purpose.

Study PKU-001

Study PKU-001 was a multinational, open-label "enrichment" study with a single treatment (sapropterin) arm. It enrolled approximately 500 PKU patients (579 screened, 490 randomized, 485 completed) ages 8 to 48, with 96% Caucasian and approximately equal numbers of male and female. Diet was not modified. Patients received sapropterin 10 mg/kg/day orally for 8 days. Blood Phe at baseline was compared to blood Phe at Day 8 in order to identify patients with $\geq$ 30% decrease. These "responders" would be eligible for enrollment in Study PKU-003.

Ninety-six patients (20%) had $\geq$ 30% decrease in blood Phe at Day 8. Of these, 89 were screened and 88 randomized in study PKU-003.

Study PKU-003

Study PKU-003 was a double-blind, randomized, multi-center, multinational trial of sapropterin 10 mg/kg/day vs. placebo. Subjects were enrolled at 15 sites in N. America (Canada, USA) and 12 sites in Europe (France, Ireland, Poland, Germany, Italy, UK).

Eighty nine patients were screened, and 88 randomized and completed dosing through Week 6. All patients met the following criteria: a $\geq$ 30% reduction in blood Phe in study 001, and screening blood level $\geq$ 600 µmol

Randomization was 1:1 placebo:sapropterin, resulting in 47 on placebo and 41 on sapropterin arm. Patients ranged in age from 8 to 49 with 98% Caucasian and a slight majority of males.

Sapropterin was administered orally once daily in the morning dissolved in 4-8 oz. of water, apple juice or orange juice. Duration of treatment was 6 weeks; diet was not controlled.

Blood levels of Phe were measured 2.5 to 5 hours after breakfast at screening, baseline visits 1 and 2, Weeks 0, 1, 2, 4, and 6. Blood Phe was measured at week 0 before patients received study drug.

The primary efficacy outcome was change in blood Phe from baseline to Week 6. Secondary outcomes include the percent of patients with blood Phe < 600 µmol at the end of Week 6.

The Week 6 change in blood Phe levels was compared between groups using an analysis of covariance model with baseline blood Phe level and treatment as covariates. The proportion of subjects who had blood Phe levels < 600 µmol at week 6 was compared between groups using a Fisher's Exact Test.

The primary and secondary endpoints were tested at a 2-sided type I error rate of 0.05. There was no adjustment for multiplicity

Study PKU-006

The overall intent of study PKU-006 was to evaluate the use of sapropterin in increasing tolerance to phenylalanine in the diet, desirable to offset the lack of protein in a controlled diet. The study was conducted in two parts. The first part served as an enrichment study to identify a group of apparent responders, who were then eligible for Part 2. Part 2 was designed to determine whether dietary phenylalanine could be safely increased while taking sapropterin. This objective was implemented by adding a phenylalanine supplement to the diet of patients whose blood Phe levels were under control and were identified as responders in Part 1.

Investigational sites were in Germany, Spain, Poland and the U.S.

Part 1 of PKU-006 was a multicenter, open-label, single (sapropterin) arm trial enrolling PKU patients with diet-controlled blood Phe levels (i.e., screening and six-month average blood Phe $\leq$ 480 µmol/L). They ranged in age from 4 to 12 years old. Ninety patients

were enrolled and treated with sapropterin 20 mg/kg/day for 8 days. Diet was not modified.

Similar to PKU-001, a response in Part 1 was defined as at least 30% decrease in blood Phe from Day 1 to Day 8 with Day 8 blood Phe $\leq$ 300 μmol/L. Responders were eligible to enroll in Part 2, corresponding to PKU-003.

Part 2 of PKU-006 was a multicenter, randomized, double-blind study of sapropterin vs. placebo. Forty-six of the 50 responders from Part 1 were randomized to either 20/mg/kg/day of sapropterin or placebo in a 3:1 ratio of drug to placebo, resulting in 34 patients on sapropterin and 12 patients on placebo. The vast majority of patients were Caucasian, a slight majority (57 and 58 %) were male, and the median age was 7 years

The duration of the study was 10 weeks. Patients were evaluated weekly either at study sites (Weeks 0, 2, 4, 6, 8 and 10) or at home (weeks 1, 3, 5, 7, 9). Diet was held at a constant low-Phe level until after week 3, when modification with a daily supplement was possible. Diet could be modified after week 3 based on current blood Phe levels.

The primary efficacy endpoint was the maximum daily Phe supplement tolerated. This is defined as the maximum amount of supplemental Phe ingested daily while maintaining blood Phe < 360 μmol/L at week 10. If the patient's blood Phe level was not in control at week 10, the tolerated amount was defined as that amount of dietary Phe supplement last ingested accompanied by a blood Phe < 360 μmol/L.

The study was designed to test within-group changes in the sapropterin arm. Comparison with placebo was considered a secondary analysis.

A key secondary endpoint was the difference in blood Phe levels between week 0 in Part 2 prior to dosing and Week 3 prior to Phe supplementation. Other secondary endpoints included bi-weekly (Weeks 3, 5, 7, and 9) daily Phe supplement prescribed.

1.3. Statistical Issues and Findings

The design and analysis of the studies are adequate for the indication and use agreed upon in labeling negotiations, namely the lowering of blood Phe in PKU patients. However, designing a study to test within-group changes in the active arm only, as was done in PKU-006, is problematic in this application. With all measurements on the primary outcome (amount of supplement tolerated) necessarily positive, chances are high of finding a significant difference from zero, the amount of supplement given prior to dosing. A comparison between sapropterin and placebo of the amount tolerated would have been more appropriate as a primary analysis. The study is likely to be underpowered for comparisons of greater interest.

The results are summarized in the following sections.

Results--Study PKU-003

At week 6, the placebo group had a mean increase of approximately 3 μmol/L in blood Phe, while the sapropterin arm decreased by 236 μmol/L.

The mean change from baseline to week 6 (adjusted for baseline blood Phe level) for the placebo group is 6 while that for the sapropterin is -245. The difference between the groups in these changes is statistically significant ($p<0.001$).

Results--Study PKU-006

For the ITT populations, the week 10 mean Phe supplement tolerated by patients receiving sapropterin was 21 mg/kg/day. This value was significantly different from the pre-treatment amount of zero supplement ($p<0.001$). Mean Phe supplement tolerated at week 10 was 25 mg/kg/day for subjects in the < 300 μmol/L blood Phe stratum and 17 mg/kg/day for those in the ≥ 300 μmol/L stratum. Twelve subjects (36%) tolerated 10 mg or less; 3 (9%) tolerated 41 to 50 mg/day (per protocol, the largest Phe supplement prescribed was 50 mg/kg/day); and 18 (55%) tolerated a Phe supplement of 11 to 40 mg/kg/day.

The week 10 mean Phe supplement tolerated by patients in the placebo group was 3 mg/kg/day, a value that the applicant states was "statistically different ($p=0.027$) from zero but not clinically meaningful" (study report, p.91). Seven placebo subjects (58%) were not able to tolerate any Phe supplement at week 10 and the other 5 (42%) tolerated only 5 or 10 mg/kg/day.

Given the final indication and the use of PKU-006 to support the findings of PKU-003, an important measure of efficacy is the difference in blood Phe levels between week 0 in Part 2 prior to dosing and week 3 prior to Phe supplementation. This within–group difference was designated a secondary efficacy outcome. The placebo group decreased from 326 μmol/L at week 0 to 230 at Week 3. The sapropterin patients decreased from 276 to 127 μmol/L at week 3. While the sapropterin group has a numerical advantage, the comparison between treatment groups was not statistically significant: the p-value was 0.32 for the one-way ANOVA model fit.

2. INTRODUCTION

2.1. Overview

Sapropterin is intended to treat phenylketonuria (PKU). Patients with PKU cannot metabolize dietary phenylalanine (Phe), an essential amino acid found in most protein sources. It is a rare disease with an incidence rate on the order of 1/12,000 live births in the U.S. PKU is caused by a deficiency in activity of the enzyme phenylalanine hydroxylase (PAH). The gene is located on chromosome 12; gene defects may result in decreased amount of normal enzyme

The usual standard of care for patients with PKU is strict dietary control via a low protein diet. The only other available treatment is liver transplant, which is considered extreme and rarely used.

Sapropterin is a new molecular entity that is structurally similar to endogenous BH4, a molecule that activates PAH during the metabolism of Phe. Sapropterin is thought to be a more energetically efficient activator of PAH than BH4, and therefore likely to be useful to patients with a decreased amount of normal enzyme.

The indication proposed by the applicant is

[redacted]

A more limited indication, the reduction of blood phenylalanine (Phe) levels in patients with hyperphenylalaninemia (HPA) due to PKU, is preferred by the FDA and was agreed to during labeling negotiations in November, 2007.

There were two pivotal trials in this application: PKU-003 and PKU-006. In addition, trial PKU-001 served primarily to identify a group of responders for enrollment in PKU-003; the first part (of two) in PKU-006 served a similar purpose. These studies are reviewed below. A dose-ranging study, PKU-004, is not discussed in this review.

2.2. Data Sources

Data and study reports were submitted electronically in CTD format and accessed through the EDR. The location was \\CDSESUB1\EVSPROD\NDA022181\0000.

3. STATISTICAL EVALUATION

3.1. Evaluation of Efficacy

3.1.1 Study PKU-001

Design

Study PKU-001 was a multinational, open-label study with a single treatment (sapropterin) arm. It enrolled approximately 500 PKU patients (579 screened, 490 randomized, 485 completed) ages 8 to 48, with 96% Caucasian and approximately equal numbers of male and female. Diet was not modified. Patients received sapropterin 10 mg/kg/day orally for 8 days. Blood Phe at baseline was compared to blood Phe at Day 8 in order to identify patients with $\geq$ 30% decrease. These "responders" would be eligible for enrollment in Study PKU-003

Results

Descriptive statistics on the primary endpoint, blood Phe levels, are given in Table 1. Figure 1 shows a histogram representing the distribution of percent change in blood Phe levels.

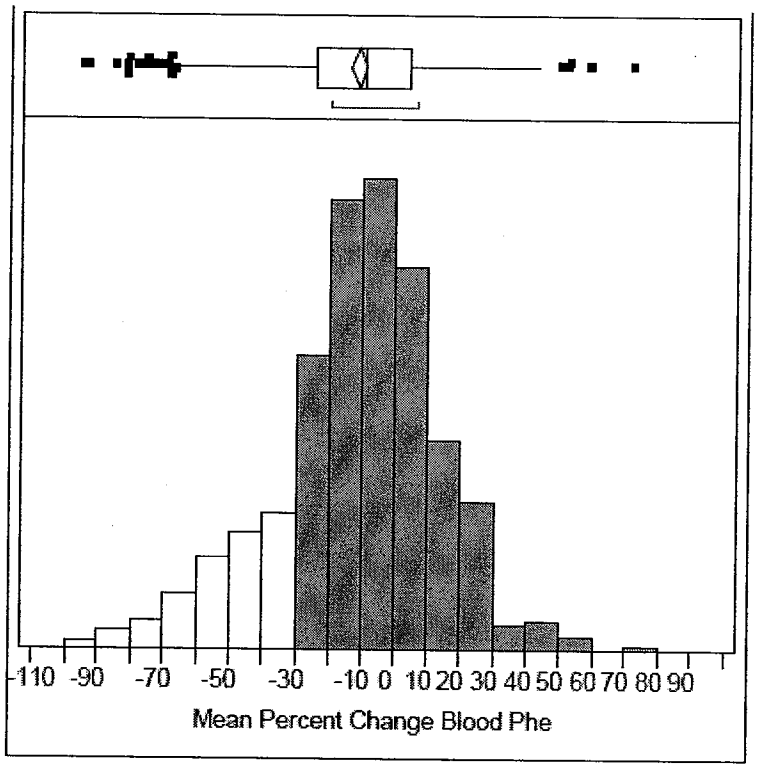
Ninety-six patients (20%) had $\geq$ 30% decrease in blood Phe at Day 8. Of these, 89 were screened and 88 randomized in study PKU-003. For the ITT population overall, blood Phe decreased 10% on average.

Table 1: Percent Change in Blood Phe Level

Blood Phe (uM)	**Day 1 Blood Phe (uM)**		
	<600	>600	Analysis population
	N=57	N=432	N=485
PRIMARY Efficacy Endpoint **Percent Change Day 1 to Day 8; Responders**			
Day 8 - Day 1 (n)	31	65	96
Mean (SD)	-55 (17)	-48 (15)	-50 (16)
Median	-51	-42	-45
Percentiles (25th,75th)	-66, -44	-57, -35	-59, -36
Range (min, max)	-95, -32	-94, -30	-95, -30
Percent Change Day 1 to Day 8; Non-Responders			
Day 8 - Day 1 (n)	26	363	389
Mean (SD)	10 (27)	-3 (16)	-2 (17)
Median	15	-5	-4
Percentiles (25th,75th)	-16, 26	-15, -6	-15, 7
Range (min, max)	-29, 59	-29, 72	-29, 72

(Source: Medical Officer's review, Table 5, p.43)

Figure 1: Mean percent Change in Blood Phe.



(Source: Medical Officer's review, figure 3, p. 44)

3.1.2 Study PKU-003

Design

Study PKU-003 was a double-blind, randomized, multi-center, multinational trial of sapropterin 10 mg/kg/day vs. placebo. Subjects were enrolled at 15 sites in N. America (Canada, USA) and 12 sites in Europe (France, Ireland, Poland, Germany, Italy, UK).

The sample size was based on calculations for the change in blood Phe from baseline to week 6. The calculations assumed a mean difference between placebo and sapropterin of 150 µmol /L, a standard deviation of 85 µmol/L and 2-sided alpha of 0.05. The calculated sample size of 80 would give 95% power to detect a difference of that magnitude.

Eighty nine patients were screened, and 88 randomized and completed dosing through Week 6. All patients met the following criteria: a $\geq$ 30% reduction in blood Phe in study 001, and screening blood level $\geq$ 600 µmol (subjects enrolled prior to protocol

amendment 2, n=19) or ≥ 450 μmol/L (subjects enrolled after protocol amendment 2, n= 70), and at least 8 years of age.

Randomization was 1:1 placebo:sapropterin, resulting in 47 on placebo and 41 on sapropterin arm. Patients ranged in age from 8 to 49 with 98% Caucasian and a slight majority of males. Demographic characteristics are shown in Table A1. Randomization was stratified by study site and screening visit blood Phe (< 600 vs. ≥ 600 μmol). Each randomization list started with a block of 2, followed by blocks of 4.

Sapropterin was administered orally once daily in the morning dissolved in 4-8 oz. of water, apple juice or orange juice. Duration of treatment was 6 weeks; diet was not controlled.

Blood levels of Phe were measured 2.5 to 5 hours after breakfast at screening, baseline visits 1 and 2, Weeks 0, 1, 2, 4, and 6. Blood Phe was measured at week 0 before patients received study drug.

"Baseline" level was defined as the mean of pretreatment measurements taken at baseline visits 1 and 2 and Week 0.

The primary efficacy outcome was change in blood Phe from baseline to Week 6. Secondary outcomes include change in weekly blood Phe from baseline at weeks 1, 2, and 4; and percent of patients with blood Phe < 600 μmol at the end of Week 6.

Analysis

The Week 6 mean change in blood Phe levels was compared using an analysis of covariance model with baseline blood Phe level and treatment as covariates. Missing data were imputed using a LOCF approach. A completer analysis was also done as a supportive analysis. However, there was only one patient who was missing data at week 6 and the results are not affected by the method of handling missing data.

The proportion of subjects who had blood Phe levels < 600 μmol at week 6 was compared between groups using a Fisher's Exact Test.

The primary and secondary endpoints were tested at a 2-sided type I error rate of 0.05. There was no adjustment for multiplicity

Because of the small number of subjects at each site and in the lower blood Phe strata, the efficacy analysis was not stratified by study site or screening blood Phe.

Results

The baseline and post-treatment blood Phe levels are shown in Table 2. These are the applicant's results, confirmed by review.

Table 2: Baseline and Post-treatment Blood Phe Levels (μmol/L), PKU-003

Blood Phe level (μmol/L)	Placebo (N = 47)	Sapropterin (N = 41)
Baseline [1]		
Mean ± SD	**888.3 ± 323.1**	**842.7 ± 299.6**
Percentiles (25th, med, 75th)	**618, 790, 1141**	**620, 862, 990**
Range (min, max)	**402, 1745**	**293, 1643**
Week 1		
Mean ± SD	**862.6 ± 345.6**	**619.9 ± 354.7**
Percentiles (25th, med, 75th)	**549, 843, 1122**	**336, 596, 777**
Range (min, max)	**199, 1766**	**184, 1603**
Week 2		
Mean ± SD	**863.2 ± 325.2**	**615.8 ± 340.2**
Percentiles (25th, med, 75th)	**626, 829, 1090**	**339, 602, 839**
Range (min, max)	**125, 1648**	**67, 1397**
Week 4		
Mean ± SD	**906.9 ± 341.4**	**587.5 ± 375.5**
Percentiles (25th, med, 75th)	**640, 850, 1088**	**288, 578, 739**
Range (min, max)	**369, 1732**	**93, 1534**
Week 6 [2]		
Mean ± SD	**891.2 ± 347.6**	**606.9 ± 377.0**
Percentiles (25th, med, 75th)	**619, 873, 1143**	**307, 526, 812**
Range (min, max)	**313, 1886**	**110, 1573**

N is the number of randomized subjects who received at least one dose of study drug.
[1] Baseline blood Phe levels were calculated as the mean of the pre-treatment measurements taken at Baseline Visits 1 and 2 and at Week 0.
[2] One subject had missing values at Week 6 and was imputed using the LOCF method.

Source: Section 5.3.5.1 of the electronic submission, study report PKU-003, Table 11.3

At week 6, the placebo group had a mean increase of approximately 3 μmol/L in blood Phe, while the sapropterin arm decreased by 236 μmol/L.

The results of the primary efficacy analysis are given in Table 3. Here the mean change from baseline to week 6 (adjusted for baseline blood Phe level) for the placebo group is 6 while that for the sapropterin is -245. The difference between the groups in these changes is statistically significant ($p<0.001$).

Table 3: Effect of Sapropterin at Week 6 – Primary Efficacy Analysis – PKU-003

	Placebo (N=47)		Sapropterin (N=41)		Effect of Sapropterin		
Model of analysis	n	Change in blood Phe level (µmol/L) mean ± SE [1]	n	Change in blood Phe level (µmol/L) mean ± SE [1]	Difference (sapropterin - placebo) mean ± SE	95% confidence interval	p-value
Primary efficacy analysis (LOCF) [2]	**47**	**6 ± 35.8**	**41**	**-239 ± 38.3**	**-245 ± 52.5**	**(-350, -141)**	**< 0.001**
Completer analysis [3]	**46**	**7 ± 36.4**	**41**	**-240 ± 38.5**	**-247 ± 53.1**	**(-353, -142)**	**< 0.001**

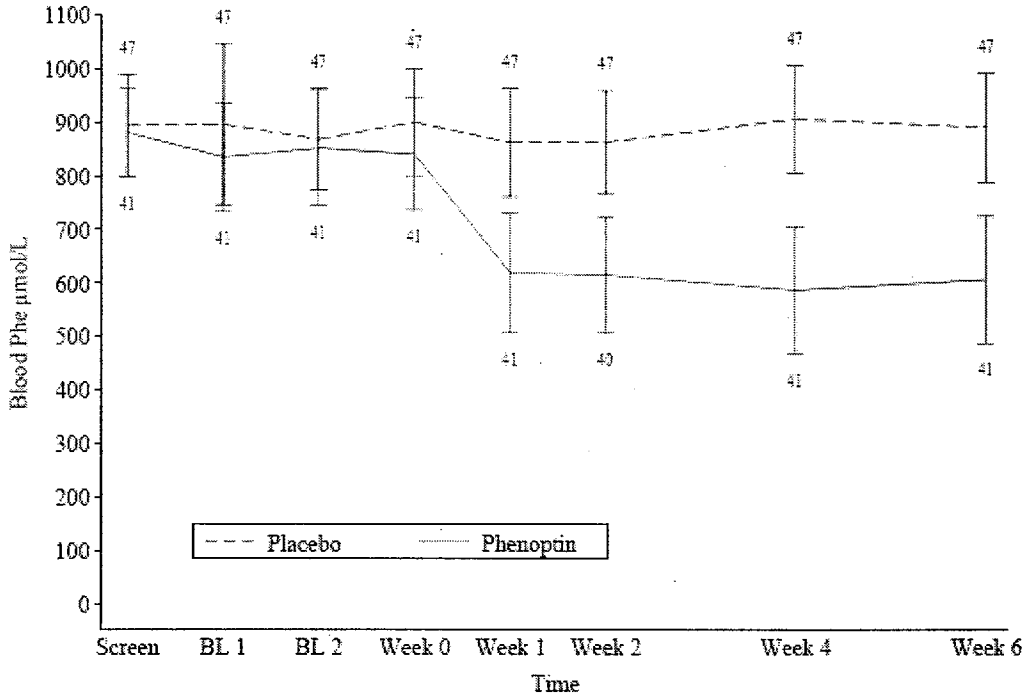
N is the number of subjects who received at least one dose of study drug, and n is the number of subjects included in each model.
[1] Least squared means and standard errors are presented.
[2] ANCOVA model with change from baseline as the response variable and both treatment group and baseline blood Phe level as covariates. A missing Week 6 blood Phe levels was imputed using LOCF for one subject.
[3] ANCOVA model with change from baseline as the response variable and both treatment group and baseline blood Phe level as covariates. Only subjects with an observed Week 6 blood Phe level were included.

Source: section *5.3.5.1* of the electronic submission; Study report PKU-003, Table 11.5

Figure 2 below shows mean blood Phe levels for each group at each visit and associated 95% CI. A separation between the groups (non-overlapping CI) is seen at the end of the first week of treatment (Week 1).

At week 6, the placebo group had a mean increase of approximately 3 µmol/L in blood Phe, while the sapropterin arm decreased by 236 µmol/L.

Figure 2: Mean Blood Phe Levels over 6 Weeks, PKU-003



Displayed are mean blood Phe values for each treatment group at each visit and the associated 95% CIs. The numbers above and below the lines are the number of subjects who have data at a given time point. BL refers to Baseline Visit.

Source: section 5.3.5.1 of the electronic submission; study report, PKU-003, p. 69

A secondary outcome was the proportion of subjects whose blood Phe level was < 600 µmol/L at Week 6. The sapropterin and placebo groups were compared using a Fisher's Exact Test for the whole cohort and for those subjects with a screening blood Phe level ≥ 600 µmol/L. At Screening, the percentage of subjects with blood Phe levels ≥ 600 µmol/L was 81% in the placebo group and 83% in the sapropterin group. At Week 6, 23% of the placebo group and 54% of the sapropterin group had achieved blood Phe levels< 600 µmol/L ($p = 0.004$).

3.1.3 Study PKU-006

Design

Study PKU-006 was conducted in two parts. The first part served as an enrichment study to identify a group of apparent responders, who were then eligible for Part 2. The overall intent of the study was to evaluate the use of sapropterin in increasing tolerance to phenylalanine in the diet, desirable to offset the lack of protein in a controlled diet. This objective was implemented in Part 2 by adding a phenylalanine supplement to the diet of patients whose blood Phe levels were under control and were identified as responders in Part 1.

Investigational sites were in Germany, Spain, Poland and the U.S.

Part 1 of PKU-006 was a multicenter, open-label, single (sapropterin) arm trial enrolling PKU patients with diet-controlled blood Phe levels (i.e., screening and six-month average blood Phe $\leq$ 480 µmol/L). They ranged in age from 4 to 12 years old. Ninety patients were enrolled and treated with sapropterin 20 mg/kg/day for 8 days. Diet was not modified.

Similar to PKU-001, a response in Part 1 was defined as at least 30% decrease in blood Phe from Day 1 to Day 8 with Day 8 blood Phe $\leq$ 300 µmol/L. Responders were eligible to enroll in Part 2, corresponding to PKU-003.

Part 2 of PKU-006 was a multicenter, randomized, double-blind study of sapropterin vs. placebo. Forty-six of the 50 responders from Part 1 were randomized to either 20/mg/kg/day of sapropterin or placebo in a 3:1 ratio of drug to placebo, resulting in 34 patients on sapropterin and 12 patients on placebo.

The randomization schedule was created using block sizes of 4. Randomization to treatment group was stratified by the average blood Phe levels in the 6 months prior to screening (<300 vs. $\geq$ 300 µmol/L).

The duration of the study was 10 weeks. Patients were evaluated weekly either at study sites (Weeks 0, 2, 4, 6, 8 and 10) or at home (weeks 1, 3, 5, 7, 9). Diet was held at a constant low-Phe level until after week 3, when modification with a daily supplement was possible. Diet could be modified after week 3 based on current blood Phe levels, as outlined in table A2 in the Appendix.

Endpoints

The primary efficacy endpoint was the maximum daily Phe supplement tolerated. This is defined as the maximum amount of supplemental Phe ingested daily while maintaining blood Phe < 360 μmol/L at week 10. If the patient's blood Phe level was not in control at week 10, the tolerated amount was defined as that amount of dietary Phe supplement last ingested accompanied by a blood Phe < 360 μmol/L.

Secondary endpoints included weekly blood Phe levels in both groups, the difference in blood Phe levels between week 0 in Part 2 prior to dosing and Week 3 prior to Phe supplementation, and bi-weekly (Weeks 3, 5, 7,and 9) daily Phe supplement prescribed. No adjustments were made for multiple secondary endpoints.

Sample Size Calculations

The protocol called for 40 responders from Part 1 to be enrolled in Part 2. The applicant assumed that a mean difference of 17.5 mg/kg/day in Phe supplement from the start of part 2 to Week 10 would be found in the sapropterin group, with a standard deviation of 16 mg/kg/day. This sample size would give an estimated power of over 99% to detect a non-zero change in Phe intake in the sapropterin group.

Analysis

The primary efficacy analysis compared the mean Phe supplement tolerated at week 10 to 0 (the amount at the start of study) using a one sample paired t-test. This analysis was done separately for each treatment group. As a secondary efficacy analysis, the applicant compared the treatment groups in the amount of Phe supplement tolerated at week 10 while maintaining adequate blood Phe control. This comparison was done using an ANCOVA model with Phe supplement tolerated at Week 10 as the outcome variable and both treatment group and blood Phe stratum as covariates.

The weekly blood Phe levels were compared between treatment groups using a repeated measures linear model. The model used treatment group and baseline blood Phe as covariates, and assumed a compound symmetry covariance structure. Baseline blood Phe was calculated as the average of the measurements at screening, day 1 of Part 1, and Week 0 of Part 2. The applicant justified the choice of compound symmetry as follows: (PKU-006 study report p. 63)

> The compound symmetry covariance structure is appropriate because the half-life of Kuvan is approximately 4.5 hours. Therefore, the correlation between the weekly blood Phe measurements should only be affected by the behavior of the subject during the previous 24 hours, not during the previous week. For example, the correlation between the Week 0 and the Week 2 blood Phe levels should be similar to the correlation between the

Week 0 and the Week 10 blood Phe levels. In addition, the small sample size (n =40) mandates selection of a covariance structure with very few elements to estimate; otherwise, the model may be overfit and hence not be relevant to a large population."

Reviewer's Comment: This argument seems sensible.

The bi-weekly total daily Phe supplement prescribed was compared between treatment groups using a similar repeated measures linear model. Here the only covariate was treatment group, and again the model assumed a compound symmetry covariance structure.

The difference in blood Phe levels between Week 0 (in Part 2) prior to dosing and Week 3 prior to diet modification for the sapropterin group was compared to the same quantity for the Placebo group This analysis was done with a 1-way analysis of variance.

The differences in blood Phe levels between week 0 in Part 2 prior to dosing and Week 3 prior to Phe supplementation in the sapropterin group were compared with the same quantities in the placebo group. This analysis was done with a 2-way analysis of covariance with blood Phe stratum as a covariate in the model. (The blood Phe stratum, which was used in the randomization, was determined by averaging the blood Phe levels in the 6 months prior to screening.) Use of this covariate was not specified in the statistical analysis plan. The applicant states that "...the omission of blood Phe stratum from the ANOVA model was subsequently determined to be an oversight because randomization was performed within blood Phe stratum."

Handling missing data

The primary efficacy outcome for Part 2 was the amount of Phe supplement tolerated at Week 10. The analysis included all subjects randomized to Part 2 who took at least one dose of study drug during Part 2. For subjects missing Phe supplement prescription data (i.e., the adjustment prescribed at Week 9), the total daily Phe supplement prescribed up until the last adjustment at which the subject was under blood Phe control (blood Phe level <360 μmol/L) was used. Forty-five of the 46 subjects randomized in Part 2 (98%) received at least one dose of study drug.

The same convention was used for calculating the amount of Phe supplement tolerated at Weeks 4, 6, and 8, so that all 45 subjects who received at least one dose of study drug during Part 2 were included in the calculation of mean Phe supplement tolerated at Weeks 4, 6, and 8.

A per-protocol analysis of the primary efficacy endpoint was restricted to subjects who completed the study and had Week 0 and all Phe supplement prescriptions recorded through Week 10. This supplementary analysis included 41 of the 45 subjects from the primary efficacy analysis.

The primary efficacy analyses used all available data from the 45 of 46 subjects randomized in Part 2 who took at least one dose of study drug during Part 2 (i.e., the ITT population). Missing data were not imputed.

Additional note

Two subjects were not under blood Phe control at week 10. The Phe supplement tolerated was the Phe supplement prescribed over all the visits prior to the visit where the subject was last observed to have blood Phe <360 μmol/L.

Thus, for these two patients, the Phe supplement value recorded at week 10 and used in the primary efficacy analysis was actually not tolerated at week 10.

Results

Demographics

Table A3 in the appendix gives the demographic characteristics for the patients in Part 1 of PKU-006 and table A4, for Part 2. The vast majority of patients were Caucasian, a slight majority (57 and 58 %) were male, and the median age was 7 years. In Part 2, the treatment groups were similar with respect to demographic characteristics, with a slight imbalance in gender distribution.

Fifty (56%) of the 89 subjects in Part 1 were classified as responders and met inclusion criteria for Part 2; 45 were randomized (12 placebo, 33 sapropterin) and received at least one dose of study drug.

Primary efficacy outcome

For the ITT population, Table 4 summarizes the primary efficacy analysis. These are the applicant's results, confirmed by review.

The Week 10 mean Phe supplement tolerated by patients receiving sapropterin was 21 mg/kg/day. This value was significantly different from the pre-treatment amount of zero supplement ($p<0.001$). Mean Phe supplement tolerated at week 10 was 25 mg/kg/day for subjects in the < 300 μmol/L blood Phe stratum and 17 mg/kg/day for those in the ≥ 300 μmol/L stratum. Twelve subjects (36%) tolerated 10 mg or less; 3 (9%) tolerated 41 to 50 mg/day (per protocol, the largest Phe supplement prescribed was 50 mg/kg/day); and 18 (55%) tolerated a Phe supplement of 11 to 40 mg/kg/day.

Table 4:
Phe Supplement Tolerated at Week 10 by Treatment Group for Part 2, PKU-006

Phe supplement tolerated (mg/kg/day)	Placebo (N= 12)	sapropterin (N= 33)
Blood Phe stratum		
< 300 µmol/L		
n	5	16
Mean ± SD	2.0 ± 4.5	24.7 ± 16.0
Percentiles (25th, med, 75th)[1]	0	13, 20, 35
Range (min, max)	0, 10	0, 50
≥ 300 µmol/L		
n	7	17
Mean ± SD	3.6 ± 3.8	17.4 ± 14.5
Percentiles (25th, med, 75th)[1]	5	5, 15, 35
Range (min, max)	0, 10	0, 40
Overall		
n	12	33
Mean ± SD	2.9 ± 4.0	20.9 ± 15.4
Percentiles (25th, med, 75th)[1]	0	10, 20, 35
Range (min, max)	0, 10	0, 50
95% confidence interval	0.4, 5.4	15.4, 26.4
p-value (one-sample t-test)[2]	0.027	< 0.001
Categories of Phe supplement tolerated, n(%)[3]		
0 mg/kg/day	7 (58)	5 (15)
1 - 10 mg/kg/day	5 (42)	7 (21)
11 - 20 mg/kg/day	0	8 (24)
21 - 30 mg/kg/day	0	2 (6)
31 - 40 mg/kg/day	0	8 (24)
41 - 50 mg/kg/day	0	3 (9)

N is the number of randomized subjects who received at least one dose of study drug during Part 2; n is the number of subjects within each blood Phe stratum and overall. Phe supplement tolerated at Week 10 is defined as the cumulative increase/decrease in Phe supplement prescribed while the subject was under blood Phe control (blood Phe < 360 µmol/L). Phe supplement tolerated at Week 10 is determined for all subjects regardless of whether they attended the Week 10 visit. 1 The sample sizes in the Placebo group are too small to estimate the 25th and 75th percentiles; therefore, only the medians are displayed. 2 The following hypotheses were used for the one-sample t-test: H0: mean is equal to 0; HA: mean is not equal to 0. 3 The number of subjects within each category of Phe supplement is indicated by n. Percentages (%) were calculated using N.

Source: Section 5.3.5.1 of electronic submission, Study report PKU-006, p. 93, Table 11.11

The week 10 mean Phe supplement tolerated by patients in the placebo group was 3 mg/kg/day, a value that the applicant states was "statistically different (p=0.027) from zero but not clinically meaningful" (study report, p.91). Seven placebo subjects (58%) were not able to tolerate any Phe supplement at week 10 and the other 5 (42%) tolerated only 5 or 10 mg/kg/day.

Results were similar for the completers (n=9 placebo; n=32 sapropterin). These results are shown in Table A5 in the Appendix.

Secondary efficacy measures

Fitting an ANCOVA model with Phe supplement as outcome and treatment group and baseline blood Phe stratum as covariates yielded a significant difference between the treatment groups (Table 5). The difference in blood Phe level between the sapropterin and placebo groups was also statistically significant in favor of sapropterin (Table 5)

Figure 3 displays the mean Phe supplement tolerated at Weeks 4, 6, 8, and 10. The sapropterin group consistently tolerates more supplement. Analysis using a longitudinal repeated measures model found a significant difference between the treatment groups (Table 5).

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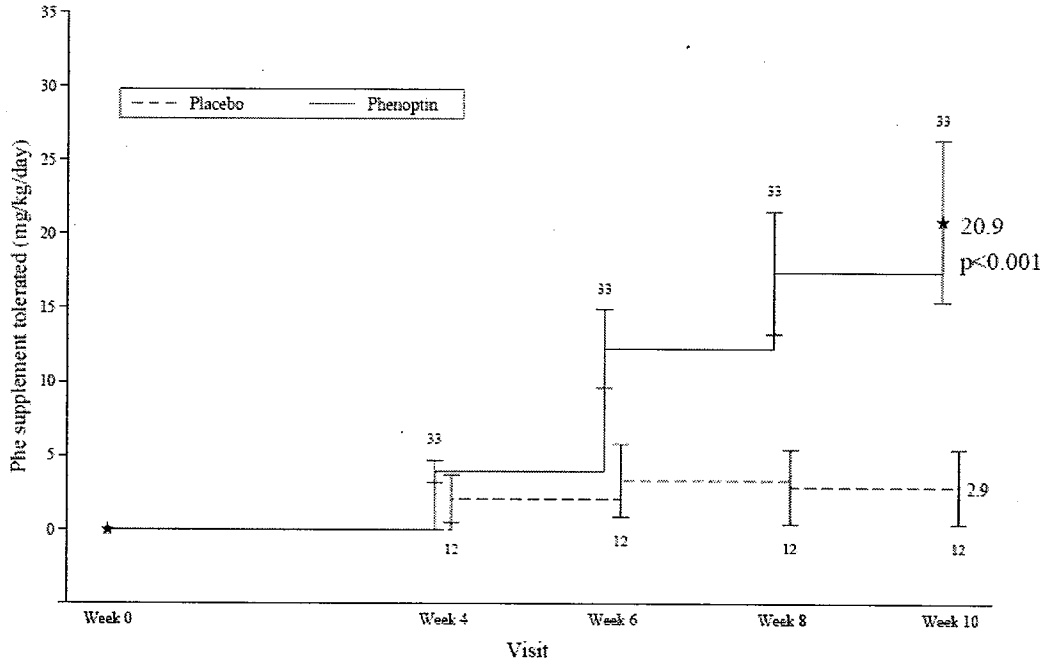
Figure 3: Phe Supplement Tolerated by Visit for Part 2, with 95% CIs



The graph presents Phe supplement tolerated and the 95% confidence intervals at Weeks 4, 6, 8, and 10.
The numbers at each visit are the number of subjects in each mean calculation.
The primary efficacy analysis compared the values indicated by a star, using a one-sample t-test.
Source: Section 5.3.5.1 of electronic submission, Study report PKU-006, p, 94, Figure 11.1

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Table 5: Effect of Sapropterin on Phe Supplement Tolerated, Blood Phe Level, and Phe Supplement Prescribed at Week 10

Response variable	Placebo (N= 12) n	Placebo (N= 12) Outcome at Week 10 mean ± SE [1]	Sapropterin (N= 33) n	Sapropterin (N= 33) Outcome at Week 10 mean ± SE [1]	Effect of Sapropterin: Difference at Week 10 (sapropterin - placebo) mean ± SE	Effect of Sapropterin: 95% confidence interval	Effect of Sapropterin: p-value
Secondary efficacy analysis							
Phe supplement tolerated [2] (mg/kg/day)	**12**	**3.3 ± 3.9**	**33**	**21.0 ± 2.3**	**17.7 ± 4.5**	**(9, 27)**	**< 0.001**
Supplemental analyses							
Blood Phe level [3] (µmol/L)	**12**	**481.2 ± 40.3**	**33**	**336.6 ± 32.2**	**-144.6 ± 33.9**	**(-213, -76)**	**< 0.001**
Phe supplement prescribed [4] (mg/kg/day)	**12**	**5.1 ± 3.0**	**33**	**22.2 ± 1.6**	**17.0 ± 3.4**	**(10, 24)**	**< 0.001**

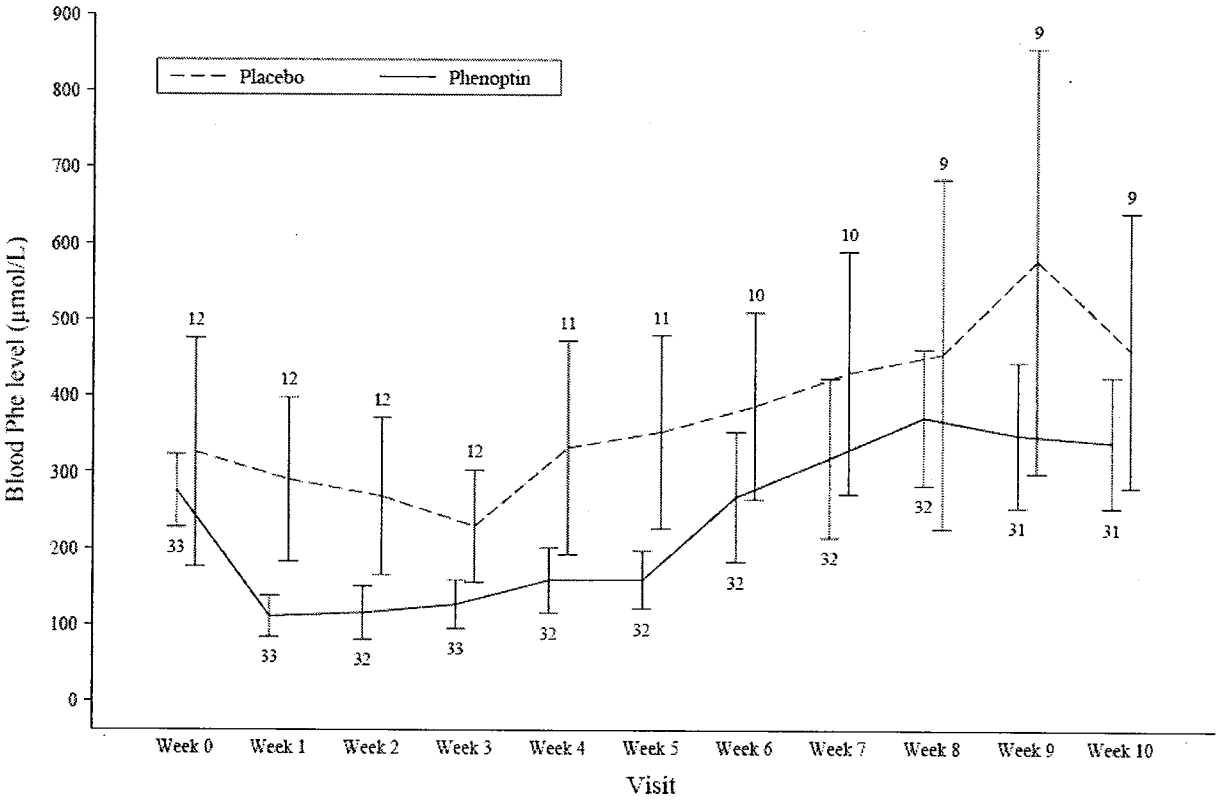
N is the number of randomized subjects who received at least one dose of study drug during Part 2; n is the number of subjects included in each model. Phe supplement tolerated at Week 10 is defined as the cumulative increase/decrease in Phe supplement prescribed while the subject was under blood Phe control (blood Phe < 360 µmol/L). Phe supplement tolerated at Week 10 is determined for all subjects regardless of whether they attended the Week 10 visit. Phe supplement prescribed at a given week is defined as the cumulative increase/decrease in Phe supplement prescribed from Week 3 until that week.

[1] Least squared means and standard errors.
[2] ANOVA model with Phe supplement tolerated at Week 10 as the outcome variable and both treatment group and blood Phe stratum as covariates.
[3] Longitudinal model with blood Phe measurements as the response variable (for Weeks 1 - 10) and treatment group, visit, and baseline blood Phe level as covariates. Baseline blood Phe is defined as the mean of Screening and Day 1 in Part 1 and Week 0 in Part 2.
[4] Longitudinal model with Phe supplement prescribed as the response variable (for Weeks 3, 5, 7, 9) and treatment group, visit, treatment by visit interaction, and blood Phe stratum as covariates. P-value for the treatment by visit interaction is < 0.001.

Source: Section 5.3.5.1 of electronic submission, Study report PKU-006, p. 115, Table 11.21

Given the final indication and the use of PKU-006 to support the findings of PKU-003, an important measure of efficacy is the difference in blood Phe levels between week 0 in Part 2 prior to dosing and week 3 prior to Phe supplementation. This within–group difference was designated a secondary efficacy outcome. Figure 4 shows the blood Phe levels by visit for Part 2. The placebo group decreased from 326 µmol/L at week 0 to 230 at Week 3. The sapropterin patients decreased from 276 to 127 µmol/L at week 3. While the sapropterin group has a numerical advantage, the comparison between treatment groups is not statistically significant: the p-value was 0.32 for the one-way ANOVA model fit.

Figure 4: Blood Phe Levels by Visit for Part 2, PKU-006, with 95% CIs



The graph presents mean blood Phe levels observed and the 95% confidence intervals at each visit.
The numbers at each visit are the number of subjects in each mean calculation.

Source: Section 5.3.5.1 of electronic submission, Study report PKU-006, p. 109

3.2. Evaluation of Safety

There were no deaths and a low incidence of adverse events in these studies. For details and further discussion, see the medical officer's review

4. FINDINGS IN SPECIAL/SUBGROUP POPULATIONS

4.1. Gender, Race, and Age

The vast majority of patients were Caucasian. Analysis by race was not done.

Males tended to have slightly greater decrease in mean blood Phe levels than females on the sapropterin arm in both studies. In PKU-003, the mean change from baseline to week 6 for males was -246, while the corresponding value for females was -216. In PKU-006, the mean change from baseline to week 3, prior to diet supplementation, was -182, while the corresponding value for females was -156.

The younger age group in each study had a greater decrease in blood Phe levels relative to older patients within the same study: In PKU-003, older patients on sapropterin (over 12 years of age) had a lower baseline but greater decrease in blood Phe levels relative to patients between the ages of 8 and 12. In PKU-006, that age group 8-12 years had a lower baseline and less reduction in blood Phe levels relative to the younger group (4-8 yrs of age) . Baseline levels are not comparable since diet was not controlled in PKU-003.

Table 6: Effects of Sapropterin in Subgroups

	Averaged baseline blood Phe and Change in blood Phe from averaged baseline (Mean ± SD, µmol/L)					
	PKU-003 (N = 41)			**PKU-006 (N = 33)**		
Subgroup	**n**	**Averaged baseline[1]**	**Change at Week 6**	**n**	**Averaged baseline[2]**	**Change at Week 3**
Age						
4 ≤ Age < 8	n/a	n/a	n/a	16	321 ± 142	-183 ± 128
8 ≤ Age ≤ 12	6	631± 193	-307 ± 297	17	278 ± 100	-161 ± 128
Age > 12	35	879± 301	-224 ± 252	n/a	n/a	n/a
Gender						
Male	27	842 ± 279	-246 ± 267	20	308 ± 126	-182 ± 124
Female	14	844 ± 347	-216 ± 246	13	285 ± 119	-156 ± 133

4.2. Other Special/Subgroup Populations

No other subgroups were identified.

5. SUMMARY AND CONCLUSIONS

5.1. Statistical Issues and Collective Evidence

The design and analysis of the studies are adequate for the indication and use agreed upon in labeling negotiations, namely the lowering of blood Phe in PKU patients. Study PKU-003 provides the primary evidence for this indication, with the flaw that diet in the study patients was not controlled. Study PKU-006 provides supportive evidence, with blood Phe levels as a secondary outcome. Although the difference was not statistically significant, sapropterin seemed to lower blood Phe levels relative to placebo prior to the increase in dietary phenylalanine after Week 3. However, designing a study (PKU-006) to test within-group changes in the active arm only is problematic in this application. With all measurements on the primary outcome (amount of supplement tolerated) necessarily positive, chances are high of finding a significant difference from zero, the amount of supplement given prior to dosing. A comparison between sapropterin and placebo of the amount tolerated would have been more appropriate as a primary analysis. Moreover, the study is likely to be underpowered for comparisons of greater interest.

Both studies PKU-003 and PKU-006 were enriched by enrolling only "responders" to sapropterin. The final label (November, 2007) reflects the use of the initial studies PKU-001 and part 1 of PKU-006 to identify responders and enrich the subsequent studies PKU-003 and PKU-006, part 2. PKU-001 identified a responder after a week on 10 mg/kg/day and PKU-006, part 1 identified a responder after a week on 20 mg/kg/day. The label reflects this design in Section 2, "Dosage and Administration," as follows:

> 2.1 Dosage
>
> The recommended starting dose of Kuvan is 10 mg/kg/day taken once daily.
>
> Response to therapy is determined by change in blood Phe following treatment with Kuvan at 10 mg/kg/day for a period of up to 1 month. Blood Phe levels should be checked after 1 week of Kuvan treatment and periodically for up to a month. If blood Phe does not decrease from baseline at 10 mg/kg/day, the dose may be increased to 20 mg/kg/day. Patients whose blood Phe does not decrease after 1 month of treatment at 20 mg/kg/day are non-responders, and treatment with Kuvan should be discontinued in these patients.
>
> Once responsiveness to Kuvan has been established, the dosage may be adjusted within the range of 5 to 20 mg/kg/day according to response to therapy. Doses of Kuvan above 20 mg/kg/day have not been evaluated in clinical trials.

5.2. Conclusions and Recommendations

The data support the conclusion that sapropterin is safe and efficacious in lowering blood Phe levels in PKU patients. There is insufficient data, in terms of number and length of studies, to indicate a change in diet for PKU patients receiving sapropterin treatment. For example, there is no information on neurological changes or developmental improvements due to long-term increased dietary phenylalanine. For further discussion see the medical officer's review.

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6. APPENDIX

The following tables are found in the Appendix:

Table A1: Demographic and Pretreatment Characteristics, Study PKU-003
Table A2: Change in Dietary Phe Supplementation Based on Blood Phe, Study PKU-006
Table A3: Demographic Characteristics for Part 1 (at Screening), Study PKU-006
Table A4: Demographic Characteristics for Part 2, Study PKU-006
Table A5: Phe Supplement Tolerated at Week 10 for Completers in Part 2, PKU-006

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Table A1: Demographic and Pretreatment Characteristics, PKU-003

Characteristic	Placebo (N = 47)	Sapropterin (N = 41)	Total (N = 88)
Gender, n (%)			
Male	**24 (51)**	**27 (66)**	**51 (58)**
Female	**23 (49)**	**14 (34)**	**37 (42)**
Age (years)			
n	**47**	**41**	**88**
Mean ± SD	**19.5 ± 9.8**	**21.5 ± 9.5**	**20.4 ± 9.7**
Percentiles (25th, med, 75th)	**13, 17, 23**	**15, 18, 29**	**14, 18, 25**
Range (min, max)	**8, 49**	**8, 42**	**8, 49**
Age category (years), n (%)			
8 ≤ Age ≤ 12	**11 (23)**	**6 (15)**	**17 (19)**
12 < Age	**36 (77)**	**35 (85)**	**71 (81)**
Race, n (%)			
Caucasian	**47 (100)**	**39 (95)**	**86 (98)**
Asian / Pacific Islander	**0**	**1 (2)**	**1 (1)**
Other	**0**	**1 (2)**	**1 (1)**
Screening blood Phe level, n (%)			
Phe < 600 µmol/L	**9 (19)**	**7 (17)**	**16 (18)**
Phe ≥ 600 µmol/L	**38 (81)**	**34 (83)**	**72 (82)**

N is the number of randomized subjects who received at least one dose of study drug. For categorical variables, n is the number of subjects with that characteristic, and percentages (%) were calculated using subjects with nonmissing data.

Source: Section 5.3.5.1 of electronic submission, Study report, PKU-003, Table 11.1

Table A2: Change in Dietary Phe Supplementation Based on Blood Phe

Blood Phe at Week 2 (uM)	Action after Week 3 blood sampling	
0 < 300	Add dietary Phe supplement (5 mg/kg/day)	
301 < 480	No change in Phe supplement intake	
481 and higher	No change in Phe supplement intake and monitor blood Phe level at the next visit	
Blood Phe at Week 4, 6, and 8 (uM)	Action at Next Visit's Blood Sampling	
0 < 180	Increase Phe supplement by 15 mg/kg/day	
181 < 240	Increase Phe supplement by 10 mg/kg/day	
241 < 300	Increase Phe supplement by 5 mg/kg/day	
301 < 359	No change in Phe supplement intake	
360 and greater	**Query: Did patient have prior Phe supplement increase(s)**	
	YES ↓	**NO** ↓
	Remove Phe supplement in reverse order, beginning with the amount of the most recent increase	No change in Phe supplement intake
481 < 1,199	• If first occasion at this level, monitor blood Phe level at the next visit • If second occasion at this level provide dietary counseling	
1,200 and greater	• If first occasion at this level, provide dietary counseling and monitor blood Phe level at the next visit • If second occasion at this level, provide dietary counseling and terminate from study drug	

Source: Medical Officer's review, p.37, Table 3.

Table A3: Demographic Characteristics for Part 1 (at Screening), Study PKU-006

Characteristic	Blood Phe stratum (μmol/L) < 300 (N = 47)	≥ 300 (N = 43)	Total (N = 90)
Gender, n (%)			
Male	**28 (60)**	**23 (53)**	**51 (57)**
Female	**19 (40)**	**20 (47)**	**39 (43)**
Age (years)			
n	**47**	**43**	**90**
Mean ± SD	**6.8 ± 2.5**	**7.8 ± 2.5**	**7.3 ± 2.5**
Percentiles (25th, med, 75th)	**5, 6, 9**	**6, 7, 10**	**5, 7, 10**
Range (min, max)	**4, 11**	**4, 12**	**4, 12**
Race, n (%)			
Caucasian	**44 (94)**	**41 (95)**	**85 (94)**
Hispanic	**1 (2)**	**0**	**1 (1)**
Native American	**1 (2)**	**0**	**1 (1)**
Other	**1 (2)**	**2 (5)**	**3 (3)**
Standing height (cm)			
n	**47**	**43**	**90**
Mean ± SD	**124 ± 16**	**129 ± 15**	**126 ± 16**
Percentiles (25th, med, 75th)	**113, 124, 136**	**116, 126, 141**	**113, 126, 139**
Range (min, max)	**96, 154**	**102, 159**	**96, 159**
Weight (kg)			
n	**47**	**43**	**90**
Mean ± SD	**26.7 ± 8.5**	**29.9 ± 9.6**	**28.2 ± 9.1**
Percentiles (25th, med, 75th)	**19, 25, 35**	**23, 26, 37**	**21, 26, 36**
Range (min, max)	**14, 47**	**16, 51**	**14, 51**
Body mass index (kg/m²)			
n	**47**	**43**	**90**
Mean ± SD	**17.0 ± 1.8**	**17.5 ± 2.2**	**17.2 ± 2.0**
Percentiles (25th, med, 75th)	**15, 17, 18**	**16, 17, 19**	**16, 17, 18**
Range (min, max)	**14, 22**	**15, 23**	**14, 23**

N is the number of subjects who received at least one dose of study drug during Part 1. For categorical variables, n is the number of subjects with that characteristic, and percentages (%) were calculated using subjects with nonmissing data. For continuous variables, only subjects with nonmissing data (n) were included in the summary of those variables.

Source: Section 5.3.5.1 of electronic submission, Study report PKU-006, p. 75, Table 11.1

Table A4: Demographic Characteristics for Part 2, Study PKU-006

Characteristic	Placebo (N = 12)	Sapropterin (N = 33)	Total (N = 45)
Gender, n (%)			
Male	**6 (50)**	**20 (61)**	**26 (58)**
Female	**6 (50)**	**13 (39)**	**19 (42)**
Age at Week 0 (years)			
n	**12**	**33**	**45**
Mean ± SD	**7.1 ± 2.0**	**7.7 ± 2.8**	**7.5 ± 2.6**
Percentiles (25th, med, 75th) [1]	**7**	**5, 8, 10**	**6, 7, 10**
Range (min, max)	**4, 10**	**4, 12**	**4, 12**
Race, n (%)			
Caucasian	**11 (92)**	**33 (100)**	**44 (98)**
Hispanic	**1 (8)**	**0**	**1 (2)**
Standing height at Screening (cm)			
n	**12**	**33**	**45**
Mean ± SD	**125 ± 12**	**128 ± 16**	**128 ± 15**
Percentiles (25th, med, 75th) [1]	**124**	**115, 131, 141**	**116, 129, 138**
Range (min, max)	**102, 149**	**100, 152**	**100, 152**
Weight at Week 0 (kg)			
n	**12**	**33**	**45**
Mean ± SD	**27.6 ± 8.0**	**30.4 ± 9.8**	**29.7 ± 9.3**
Percentiles (25th, med, 75th) [1]	**25**	**22, 29, 37**	**22, 27, 37**
Range (min, max)	**17, 44**	**17, 52**	**17, 52**
Body mass index [2] (kg/m^2)			
n	**12**	**33**	**45**
Mean ± SD	**17.3 ± 2.2**	**17.9 ± 2.3**	**17.8 ± 2.3**
Percentiles (25th, med, 75th) [1]	**17**	**16, 18, 19**	**16, 17, 19**
Range (min, max)	**15, 22**	**14, 24**	**14, 24**

N is the number of randomized subjects who received at least one dose of study drug during Part 2. For categorical variables, n is the number of subjects with that characteristic, and percentages (%) were calculated using subjects with nonmissing data. For continuous variables, only subjects with nonmissing data (n) were included in the summary of those variables.

[1] The sample sizes in the Placebo group are too small to estimate the 25th and 75th percentiles; therefore, only the medians are displayed.
[2] Body mass index was calculated using weight at Week 0 and height at Screening.

Source: Section 5.3.5.1 of electronic submission, Study report PKU-006, p. 80, Table 11.4

Table A5: Phe Supplement Tolerated at Week 10 for Completers in Part 2 , PKU-006

Phe supplement tolerated (mg/kg/day)	**Placebo (N= 9)**	**Sapropterin (N= 32)**
Blood Phe stratum		
< 300 µmol/L		
n	3	16
Mean ± SD	0.0 ± 0.0	24.7 ± 16.0
Percentiles (25th, med, 75th)[1]	0	13, 20, 35
Range (min, max)	0, 0	0, 50
≥ 300 µmol/L		
n	6	16
Mean ± SD	4.2 ± 3.8	18.4 ± 14.2
Percentiles (25th, med, 75th)[1]	5	8, 18, 35
Range (min, max)	0, 10	0, 40
Overall		
n	9	32
Mean ± SD	2.8 ± 3.6	21.6 ± 15.2
Percentiles (25th, med, 75th)[1]	0	10, 20, 35
Range (min, max)	0, 10	0, 50
95% confidence interval	0.0, 5.6	16.1, 27.0
p-value (one-sample t-test)[2]	0.051	< 0.001
Categories of Phe supplement tolerated, n(%)[3]		
0 mg/kg/day	5 (56)	4 (13)
1 - 10 mg/kg/day	4 (44)	7 (22)
11 - 20 mg/kg/day	0	8 (25)
21 - 30 mg/kg/day	0	2 (6)
31 - 40 mg/kg/day	0	8 (25)
41 - 50 mg/kg/day	0	3 (9)

N is the number of randomized subjects who received at least one dose of study drug during Part 2, received all four Phe supplement prescriptions (Weeks 3, 5, 7, and 9), and attended the Week 10 visit (i.e., Completers); n is the number of Completers within each blood Phe stratum and overall. Phe supplement tolerated at Week 10 is defined as the cumulative increase/decrease in Phe supplement prescribed while the subject was under blood Phe control (blood Phe < 360 µmol/L). 1 The sample sizes in the Placebo group are too small to estimate the 25th and 75th percentiles; therefore, only the medians are displayed. 2 The following hypotheses were used for the one-sample t-test: H0: mean is equal to 0; HA: mean is not equal to 0. 3 The number of subjects within each category of Phe supplement is indicated by n. Percentages (%) were calculated using N.

Source: Section 5.3.5.1 of electronic submission, Study report PKU-006, p. 180, Table 14.2.3

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