Efficacy of sapropterin dihydrochloride (tetrahydrobiopterin, 6R-BH4) for reduction of phenylalanine concentration in patients with phenylketonuria: a phase III randomised placebo-controlled study

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Summary

Background Early and strict dietary management of phenylketonuria is the only option to prevent mental retardation. We aimed to test the efficacy of sapropterin, a synthetic form of tetrahydrobiopterin (BH4), for reduction of blood phenylalanine concentration.

Methods We enrolled 89 patients with phenylketonuria in a Phase III, multicentre, randomised, double-blind, placebo-controlled trial. We randomly assigned 42 patients to receive oral doses of sapropterin (10 mg/kg) and 47 patients to receive placebo, once daily for 6 weeks. The primary endpoint was mean change from baseline in concentration of phenylalanine in blood after 6 weeks. Analysis was on an intention-to-treat basis. The study is registered with ClinicalTrials.gov, number NCT00104247.

Findings 88 of 89 enrolled patients received at least one dose of study drug, and 87 attended the week 6 visit. Mean age was 20 (SD 9.7) years. At baseline, mean concentration of phenylalanine in blood was 843 (300) µmol/L in patients assigned to receive sapropterin, and 888 (323) µmol/L in controls. After 6 weeks of treatment, patients given sapropterin had a decrease in mean blood phenylalanine of 236 (257) µmol/L, compared with a 3 (240) µmol/L increase in the placebo group (p<0.0001). After 6 weeks, 18/41 (44%) patients (95% CI 28–60) in the sapropterin group and 4/47 (9%) controls (95% CI 2–20) had a reduction in blood phenylalanine concentration of 30% or greater from baseline. Blood phenylalanine concentrations fell by about 200 µmol/L after 1 week in the sapropterin group and this reduction persisted for the remaining 5 weeks of the study (p<0.0001). 11/47 (23%) patients in the sapropterin group and 8/41 (20%) in the placebo group experienced adverse events that might have been drug-related (p=0.80). Upper respiratory tract infections were the most common disorder.

Interpretation In some patients with phenylketonuria who are responsive to BH4, sapropterin treatment to reduce blood phenylalanine could be used as an adjunct to a restrictive low-phenylalanine diet, and might even replace the diet in some instances.

Introduction One of the most compelling and repeated stories in medical genetics is the effective prevention of mental retardation from phenylketonuria by newborn screening and early dietary treatment to reduce the concentration of phenylalanine in blood.4

One novel approach to treatment is to enhance the activity of residual PAH by treatment with pharmacological amounts of its cofactor, tetrahydrobiopterin (BH4), or its biologically active synthetic form 6R-BH4 (sapropterin dihydrochloride, known as sapropterin). Since 1999, studies have shown that a subset of patients with phenylketonuria respond to treatment with BH44–6 and that this effect is caused by increased oxidation of phenylalanine.4 This responsiveness to BH4 is probably associated with mutations in the PAH gene that encode a variant form of the enzyme with some residual activity; however, the genotype-phenotype correlation is weak.5 In some patients with phenylketonuria, 6R-BH4 treatment has been shown to increase the tolerance for phenyl-
alanine sufficiently to allow a less restrictive diet\(^a\) or even, in some cases, discontinuation of the diet.\(^{19,20}\)

We aimed to assess the efficacy of sapropterin compared with placebo, for reduction of phenylalanine in blood in patients with phenylketonuria. Our secondary objective was to assess the safety of sapropterin compared with placebo.

**Methods**

**Participants**

We enrolled patients between March, 2005, and February, 2006, at 16 centres in North America and 14 centres in six countries in Europe. The protocol (PKU-003) was developed in collaboration with a Phenylketonuria Advisory Committee and approved at each centre by its institutional review board, research ethics board, or ethics committee. Each patient, or a parent or guardian for those aged younger than 18 years, gave written informed consent for participation in the study.

Figure 1 shows that we screened 89 patients with phenylketonuria who had relaxed or abandoned a strict low-phenylalanine diet and who had participated in a previous phase-1 screening study (Protocol PKU-001).\(^{21}\) Eligibility criteria included responsiveness in PKU-001, which was defined as a reduction of 30% or more in blood phenylalanine concentration after 8 days of treatment with sapropterin at a dose of 10 mg/kg per day.

We started to screen patients 6 weeks before randomisation. Eligibility criteria for our study were blood phenylalanine of 600 \(\mu\)mol/L or greater (or \(\geq 450 \mu\)mol/L after a protocol amendment) at the screening visit, age of 8 years or older, and willingness and ability to comply with study procedures and to adhere to their current diet. Women with childbearing potential had to have a negative urine pregnancy test to be eligible, and sexually active men and women had to adopt acceptable birth control measures to prevent pregnancy.

**Procedures**

We took two baseline measurements of blood phenylalanine concentration at 1 and 2 weeks before randomisation. At week 0, we randomised all eligible patients in a 1:1 ratio. We used an interactive voice-response telephone system to maintain blinding. Patients were stratified by study centre and by blood phenylalanine at screening visit (<600 \(\mu\)mol/L and \(\geq 600 \mu\)mol/L). Patients in the treatment group were allocated to receive 10 mg/kg sapropterin, and controls were given a placebo (which was similar in taste and appearance), orally once daily for 6 weeks.

We obtained randomisation lists from Statistics Collaborative (Washington, DC, USA); they were generated by a computer program that was verified for accuracy with strict quality-control procedures. Each randomisation list started with a block of two, followed by blocks of four, which ensured that the first two assignments in each stratum were to both the sapropterin and placebo groups, and that subsequent assignments in each stratum consisted of two sapropterin and two placebo assignments in random order.

Block size was not divulged to the study sponsor or to the study investigators until the study was completed. Investigators, patients, and sponsors were kept unaware of the treatment allocation until the database was locked.

Sapropterin and placebo tablets were dissolved in 120–240 mL of water, apple juice, or orange juice. Patients were instructed to continue their usual diet without modification. The concentration of blood phenylalanine was measured 2.5–5 h after breakfast at the screening assessment, each of the two baseline assessments, and treatment weeks 0, 1, 2, 4, and 6. We also measured the phenylalanine concentration in blood at the final visit of any patient who withdrew from the study early. Blood had been collected for assessment of \(\text{PAH}\) genotype during the screening study (PKU-001).

A central laboratory measured the concentration of phenylalanine in blood with the aminoacid analyser method. Centres and patients were forbidden to obtain such concentrations independently. Neither investigators nor patients were aware of postbaseline blood phenylalanine concentrations during the study. We also recorded medical histories, monitored adverse events and vital signs, and did physical examinations, thyroid function tests (thyroxine \([T4]\) and thyroid-stimulating hormone \([TSH]\)), and clinical laboratory tests (including chemistry, haematology, and urinalysis).

The primary endpoint, to measure efficacy of sapropterin compared with placebo, was the change in blood phenylalanine concentration from baseline to week 6. Secondary endpoints were changes in phenylalanine concentrations in blood at each of the 6 weeks of treatment, and the proportion of patients who had blood
phenylalanine of less than 600 µmol/L at week 6. To assess safety, we also compared the frequencies of all adverse events and serious adverse events in the sapropterin and placebo groups. Adverse events were classified according to the Medical Dictionary for Regulatory Activities.22

Statistical analysis
We analysed all randomised patients who received at least one dose of study drug. Based on the results of a previous pilot study,23 we assumed a difference between treatment groups in mean change at week 6 of 150 (SD 85) μmol/L, and a two-sided type I error rate of 0.05. Therefore, we calculated that a sample size of 80 patients (40 in each group) would provide over 95% power to detect a significant difference between treatment groups at week 6.

To assess change in blood phenylalanine concentration from baseline to week 6, we defined baseline blood phenylalanine as the average of two baseline assessments (1 and 2 weeks before randomisation) and the week 0 assessment. If the week 6 assessment of blood phenylalanine was missing, we used a last observation carried forward method to impute the data. We compared the mean change in blood phenylalanine concentration at week 6 with an analysis of covariance model, using change in blood phenylalanine from baseline to week 6 as the outcome variable, and treatment group as a categorical covariate.

We compared the mean change in blood phenylalanine concentration at week 6 by analysis of covariance, with change in blood phenylalanine from baseline to week 6 as the outcome variable, treatment group as a categorical covariate, and baseline blood phenylalanine concentration as a continuous covariate. We used Fisher’s exact test to compare the proportion of patients whose blood phenylalanine concentration was less than 600 µmol/L at week 6. We also did a post-hoc analysis of the proportion of patients with a blood phenylalanine of less than 360 µmol/L at week 6. We analysed data with SAS statistical software (version 9.1.3). The study is registered with ClinicalTrials.gov, number NCT00104247.

Role of the funding source
The study protocol was drafted and developed by the study sponsor, BioMarin Pharmaceuticals, in collaboration with Merck Serono. The study sponsor collected and analysed the data. All authors had full access to all data and statistical analyses in this study and participated in writing the manuscript. The corresponding author had final responsibility for the decision to submit for publication.

Results
42 patients were randomly assigned to receive sapropterin, and 47 to placebo (figure 1). Sex was the only baseline demographic for which the treatment groups differed (table 1). Treatment compliance was high; 72/88 (82%) patients took all doses of the study drug correctly throughout the 6 weeks of the study. Numbers of missed doses or dosing errors were similar in the two treatment groups. Dietary compliance was also high; only 7/41 (17%) patients in the sapropterin group and 12/47 (26%) controls reported that their diet contained an increased or decreased intake of phenylalanine. None of these deviations from protocol were regarded as serious.

Concentrations of blood phenylalanine for each patient during the 6 weeks of the study are shown in figure 2. Table 1 shows that mean blood phenylalanine concentration at baseline was 842·7 (SD 299·6) µmol/L for the Sapropterin treatment group (n=41), 843·6 (SD 299·5) µmol/L for the Placebo group (n=47), and 843·1 (SD 299·5) µmol/L for the Total (n=88).

Figure 2: Blood phenylalanine concentrations at baseline and week 6
Black line shows no change from baseline; dotted line shows 30% reduction.

Data are n (%) or mean (SD). For categorical variables, percentages include all patients with available data. For continuous variables, all patients with available data are included.
sapropterin group and 888·3 (323·1) µmol/L for controls. At 6 weeks, 18/41 (44%) patients given sapropterin (95% CI 28–60) and 4/47 (9%) controls (95% CI 2–20) had a blood phenylalanine reduction of 30% or more from baseline (p=0·0002). Blood phenylalanine concentration was reduced by 50% or more in 13/41 (32%) patients who received sapropterin (95% CI 18–48) and 1/47 (2%) controls (95% CI 0–11).

Figure 4 shows that mean blood phenylalanine in controls fluctuated only slightly from baseline over the 6 weeks of the study. By contrast, in the sapropterin group, the mean fell from 842·7 (299·6) µmol/L at baseline to 619·9 (354·7) µmol/L at week 1 and remained at this lower concentration for the duration of the study. At week 6, the mean change in blood phenylalanine from baseline for the sapropterin group was −235·9 (257·0) µmol/L, compared with 2·9 (239·5) µmol/L for controls (p<0·0001). By week 6, the mean blood phenylalanine concentration was 606·9 (377·0) µmol/L in the sapropterin group. The estimated difference between treatment groups in mean change in concentration of blood phenylalanine from baseline at week 6 was −245 (52·5) µmol/L (p=0·0002).

Table 2 shows analyses of the efficacy of sapropterin at week 6. 7/41 (17%) patients assigned to sapropterin had blood phenylalanine of less than 600 µmol/L at screening; this proportion had increased to 22/41 (54%, 95% CI 38–69) after 6 weeks (p=0·004). In controls, this proportion was 9/47 (19%) at screening and 11/47 (23%, 95% CI 11–36) at week 6. 15/34 (44%) of patients assigned
to sapropterin and 4/38 (11%) of controls had blood phenylalanine of 600 μmol/L or more at screening, and of less than 600 μmol/L at week 6 (p=0.003). 13/41 (32%) in the sapropterin group and 1/47 (2%) controls had blood phenylalanine concentrations of less than 360 μmol/L at week 6 (p<0.001).

11/47 (23%) patients in the sapropterin group and 8/41 (20%) patients in the control group experienced adverse events that might have been drug-related (p=0.80). No patient withdrew from the study because of adverse events. Most adverse events were deemed to be unrelated to the study drugs. The most commonly reported adverse event was upper respiratory tract infection. Nervous system disorders (eg, headache) were more frequent in the placebo group than in the sapropterin group (table 3). No serious adverse events were recorded in either group, and no patient died during the study.

No patients in the sapropterin group and two controls had clinically significant changes in liver enzymes (alanine aminotransferase in one and aspartate transaminase in the other). One patient in the sapropterin group had a clinically significant low T4 at week 0 (before sapropterin exposure) and again at week 6. This patient had normal TSH concentration at week 0 and high TSH after 6 weeks.

Full PAH genotypes, obtained for the PKU-001 trial, were available for 17 of the 19 patients with a reduction of 30% or more in blood phenylalanine in response to sapropterin. In one patient, only a single mutation was identified and in the other a genotype was not available. Responsiveness to BH4 is thought to require at least one PAH mutation that allows for residual enzyme activity (non-null mutation).16 17 fully genotyped patients had at least one non-null mutation, as did the patient with only one identified mutation. However, responsiveness was not consistently linked with specific mutations; six mutations were associated with both responsiveness and non-responsiveness. Moreover, the two PAH mutations identified in a patient who had a 63% reduction in blood phenylalanine after 6 weeks of sapropterin treatment were IVS10-3C→T and G272X, neither of which would seem to allow for residual PAH activity.

Discussion
This 6-week study showed that sapropterin treatment can reduce the concentration of phenylalanine in blood in some patients with phenylketonuria. Mean blood phenylalanine fell in the sapropterin group at week 1 and remained lower than in patients in the placebo group for the duration of the study. Sapropterin treatment over a 6-week period was safe. Our results suggest that sapropterin treatment might be used as an adjunct to the restrictive low-phenylalanine diet in patients with phenylketonuria, and could even replace the diet in some instances.17 These findings accord with those of earlier pilot studies that also described substantial reductions in blood phenylalanine in patients with phenylketonuria who were given oral doses of 5–20 mg/kg per day of 6R-BH4, and were able to normalise their diet.19,20,24–26

The proportion of patients assigned to receive sapropterin who had decreases in blood phenylalanine concentrations after 6 weeks of at least 30% was larger than that in controls (44% vs 9%). However, almost the same number of patients in each group, 12 given sapropterin and 10 given placebo, had an 11–29% decrease in blood phenylalanine concentration at week 6. Moreover, 7 (17%) patients given sapropterin and 21 (45%) controls had increased phenylalanine in blood. Some variations in blood phenylalanine could possibly have been caused by chance, or by undisclosed changes in dietary phenylalanine. Nevertheless, the much higher proportion of patients who had a blood phenylalanine decrease of at least 30% in the sapropterin group, compared with controls, suggests that the cause was a sapropterin response rather than a dietary difference. All patients with phenylketonuria who were enrolled in our study had prestudy reductions of at least 30% in blood phenylalanine while taking sapropterin. However, not all were necessarily truly responsive to BH4. Therefore, patients in our study for whom sapropterin treatment did not reduce blood phenylalanine might not have been BH4-responsive.

### Table 2: Analysis of efficacy of sapropterin at week 6

<table>
<thead>
<tr>
<th></th>
<th>Placebo group (n=47)</th>
<th>Sapropterin group (n=41)</th>
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<tbody>
<tr>
<td></td>
<td>Patients</td>
<td>Events</td>
<td>Patients</td>
</tr>
<tr>
<td>Any adverse events on or after first dose</td>
<td>34 (72%)</td>
<td>95</td>
<td>21 (51%)</td>
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<tr>
<td>Adverse events that occurred in 5% or more of patients</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Upper respiratory tract infection</td>
<td>13 (28%)</td>
<td>13</td>
<td>7 (17%)</td>
</tr>
<tr>
<td>Headache</td>
<td>7 (15%)</td>
<td>10</td>
<td>4 (10%)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>4 (9%)</td>
<td>4</td>
<td>2 (5%)</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>4 (9%)</td>
<td>4</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>3 (6%)</td>
<td>3</td>
<td>2 (5%)</td>
</tr>
<tr>
<td>Pyrexia</td>
<td>2 (4%)</td>
<td>2</td>
<td>2 (5%)</td>
</tr>
<tr>
<td>Back pain</td>
<td>3 (6%)</td>
<td>3</td>
<td>1 (2%)</td>
</tr>
</tbody>
</table>

Only adverse events with onset on or after first dose are summarised here. * A patient was counted at most once for a given adverse event. † Several events were counted if patients had the same adverse events with different onset dates or times.

### Table 3: Adverse effects
14/17 mutations we identified have been previously linked to a BH4-responsive phenotype;27 two mutations, G218V and Q226H, have not been reported in response patients. BH4-responsiveness is thought to occur only when at least one PAH mutation allows for residual enzyme activity. However, one patient in our study, who had a 63% decrease in concentration of blood phenylalanine while taking sapropterin, had two PAH mutations, IVS10-3C→T and G272X, both of which would seem to be null. Because two intronic mutations have been linked with BH4-responsiveness,27 the intronic mutation IVS10-3C→T could possibly be associated with residual PAH activity, and thus responsiveness. If so, the explanation could lie in alternative splicing, which would produce enzyme activity.28

Despite these genotype–phenotype associations, BH4-responsiveness cannot reliably be predicted from PAH genotype,14–17 and must therefore be determined on the basis of blood phenylalanine in response to a loading dose of BH4. The most commonly used assessment method consists of administration of two 20 mg/kg doses of 6R-BH4, 24 h apart, followed by blood phenylalanine measurements at 0, 4, 8, 12, 24, and 48 h after the first dose.18 A reduction in blood phenylalanine concentration of 30% or more at any of these timepoints is usually regarded as indicative of responsiveness, although a lesser decrease could also be therapeutically beneficial.

Several short-term dietary-intervention crossover studies in patients with early treated, well controlled phenylketonuria, and with IQs in the normal range, have shown that executive function and higher cognitive function deteriorate when the concentration of phenylalanine in blood exceeds 600 µmol/L and improve when phenylalanine is reduced to 600 µmol/L or below, even above 10 years of age.16–18 In our study, more than half the patients in the sapropterin group had blood phenylalanine concentrations of 600 µmol/L or greater by week 6. Smith and colleagues showed a 4–10 point reduction in IQ, dependent on age, at 4–10 years of age.16 Together, these findings emphasise the importance of maintaining low blood phenylalanine concentrations at all times, and support the use of sapropterin to lower blood phenylalanine in BH4-responsive patients with phenylketonuria. Furthermore, lifelong, reliable control of blood phenylalanine could also promote better overall central nervous system development and function, and alleviate the burden of a limited dietary regime.

Contributors
HLL drafted the manuscript. All authors have read and approved the manuscript and contributed to the study design, analysis, or interpretation of data, and the drafting and revision of the manuscript.

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Conflict of interest statement
HLL serves on the PKU Advisory Board and is a consultant to BioMarin Pharmaceuticals. JDB and HC-S have received funding from BioMarin Pharmaceuticals for statistical analysis. AD is an employee of BioMarin Pharmaceuticals. JDB and HC-S have received funding from BioMarin Pharmaceuticals for statistical analysis. AD is an employee of BioMarin Pharmaceuticals.

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References


