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ORIGINAL ARTICLE

# The response of patients with phenylketonuria and elevated serum phenylalanine to treatment with oral sapropterin dihydrochloride (6*R*-tetrahydrobiopterin): a phase II, multicentre, open-label, screening study

**B. K. Burton · D. K. Grange · A. Milanowski · G. Vockley · F. Feillet · E. A. Crombez · V. Abadie · C. O. Harding · S. Cederbaum · D. Dobbelaere · A. Smith · A. Dorenbaum**

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**Summary** This study aimed to evaluate the response to and safety of an 8-day course of sapropterin dihydrochloride (6*R*-tetrahydrobiopterin or 6*R*-$BH_4$) 10 mg/kg per day in patients with phenylketonuria (PKU), who have elevated blood phenylalanine (Phe) levels, and to identify a suitable cohort of patients who would respond to sapropterin dihydrochloride treatment with a reduction in blood Phe level. Eligible patients were aged ≥8 years, had blood Phe levels ≥450 μmol/L and were not adhering to a Phe-restricted diet. Suitable patients were identified by a ≥30% reduction in blood Phe level from baseline to day 8 following sapropterin dihydrochloride treatment. The proportion of patients who met these criteria was calculated for the overall population and by baseline

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*References to electronic databases:* Phenylketonuria (PKU): OMIM 261600.

B. K. Burton
Children's Memorial Hospital,
Northwestern University
Feinberg School of Medicine,
Chicago, IL, USA

D. K. Grange
St. Louis Children's Hospital,
Washington School of Medicine,
St. Louis, MO, USA

A. Milanowski
Instytut Matki i Dziecka, Warszawa, Poland

G. Vockley
Children's Hospital of Pittsburgh,
Pittsburgh, PA, USA

F. Feillet
Department of Pediatrics,
Hôpital d'Enfants,
Vandoeuvre les Nancy, France

E. A. Crombez
David Geffen School of Medicine at UCLA,
Los Angeles, CA, USA

V. Abadie
Hôpital Necker – Enfants Malades Pediatrie Generale,
Paris, France

C. O. Harding
Oregon Health & Science University,
Portland, OR, USA

S. Cederbaum
UCLA Medical Center, Los Angeles, CA, USA

D. Dobbelaere
CHRU de Lille – Hôpital Jeanne de Flandres,
Lille, France

A. Smith
Pacific Data Designs, San Francisco, CA, USA

A. Dorenbaum
BioMarin Pharmaceutical Inc., Novato, CA, USA

B. K. Burton (✉)
Children's Memorial Hospital, Division of Genetics,
Box 59, 2300 Children's Plaza, Chicago,
IL 60614, USA
e-mail: bburton@childrensmemorial.org

Phe level (<600, 600 to <900, 900 to <1200 and ≥1200 μmol/L). In total, 485/490 patients completed the study and 20% (96/485) were identified as patients who would respond to sapropterin dihydrochloride. A reduction in Phe level was observed in all subgroups, although response was greater in patients with lower baseline Phe levels. Wide variability in response was seen across all baseline Phe subgroups. The majority of adverse events were mild and all resolved without complications. Sapropterin dihydrochloride was well tolerated and reduced blood Phe levels across all PKU phenotypes tested. Variability in reduction of Phe indicates that the response to sapropterin dihydrochloride cannot be predicted by baseline Phe level.

**Abbreviations**

| | |
|---|---|
| AE | adverse event |
| $BH_4$ | tetrahydrobiopterin |
| CI | confidence interval |
| HPA | hyperphenylalaninaemia |
| MedDRA | Medical Dictionary for Regulatory Activities |
| PAH | phenylalanine hydroxylase |
| Phe | phenylalanine |
| PKU | phenylketonuria |

## Introduction

Phenylketonuria (PKU; OMIM 261600) is an autosomal, recessive metabolic disease caused by mutations in the gene encoding phenylalanine hydroxylase (PAH). The resulting PAH deficiency prevents hepatic conversion of phenylalanine (Phe) to tyrosine and leads to hyperphenylalaninaemia (HPA) (Scriver and Kaufman 2001). More than 500 mutations in the PAH gene have been identified (http://www.pahdb.mcgill.ca/), corresponding to a wide range of clinical phenotypes that vary in severity from mild HPA to classic PKU. Untreated HPA is neurotoxic and results in profound neurocognitive and developmental defects (Pietz 1998).

No specific medical therapy is available for the treatment of PKU and metabolic control is achieved through a Phe-restricted diet. If implemented from an early age, strict dietary measures can lead to good neurological outcomes (Holtzman et al 1986; Michals et al 1988; National Institutes of Health Consensus Development Conference Statement 2001). However, severely restrictive diets present a substantial psychosocial burden and are associated with a risk of nutritional deficiencies (Walter et al 2002). Furthermore, dietary treatment requires close monitoring of Phe levels, and adolescents and young adults often fail to comply with the strict recommendations (Walter et al 2002). Non-dietary treatments for PAH deficiency would, therefore, provide a valuable therapeutic option for patients with PKU and their families (National Institutes of Health Consensus Development Conference Statement 2001).

Tetrahydrobiopterin ($BH_4$) is an endogenous cofactor for PAH, the enzyme that hydroxylates Phe to tyrosine (Kaufman 1986). Data suggest that $BH_4$ and its stereoisomer, 6*R*-$BH_4$ (6*R*-tetrahydrobiopterin or sapropterin dihydrochloride, hereafter referred to as sapropterin), may reduce Phe levels in many patients with PKU (Bernegger and Blau 2002; Kure et al 1999; Matalon et al 2005; Muntau et al 2002; Shintaku et al 2003). Various mechanisms by which therapeutic $BH_4$ and 6*R*-$BH_4$ may alter Phe metabolism in patients with PKU have been described, including upregulation, activation, stabilization and modification of the tertiary structure of PAH (Blau and Erlandsen 2004; Kure et al 1999). We conducted this screening study to identify a suitable cohort of patients with PKU who respond to a short course of sapropterin, for subsequent enrolment into a long-term phase III clinical efficacy and safety trial. Here, we report efficacy and safety data from the initial screening study, including an evaluation of the impact of baseline blood Phe level on response to sapropterin.

## Methods

This phase II, open-label screening study (protocol number PKU-001) was designed to evaluate the safety of sapropterin, and degree and frequency of response to sapropterin in patients with PKU and elevated Phe levels. The study was carried out in 30 centres in North America and Europe, and was approved by local institutional review boards or ethics committees. Written, informed personal or parental consent was obtained for all patients and the study was conducted in accordance with Good Clinical Practice, the Declaration of Helsinki and the US Code of Federal Regulations.

### Patients

Patients with PKU were eligible for inclusion in the study if they were aged ≥8 years, had a previously documented Phe level ≥360 μmol/L and a Phe level ≥450 μmol/L at the screening visit. Patients participating in the trial were those who were not adhering to the strict low-Phe diet recommended for the

management of PKU. Although dietary compliance is strongly encouraged for all age groups, it is more likely to be enforced in younger patients through parental control, leading to a lower rate of noncompliance than in older patients, as reflected in the age distribution of patients included in this study. Nonadherence to a strict low-Phe diet was documented prior to study entry by the presence of elevated Phe levels. Participants were instructed to continue their usual dietary intake pattern during the study and were asked to report any changes in diet at the day-8 visit. Information on the benefit of dietary compliance as part of standard care was provided to all participants on day 8 and at study termination. The minimum age of 8 years was chosen because it was considered inappropriate to instruct patients in the younger age groups with poorly controlled diets to make no changes to their diet during the duration of the study. Patients with a diagnosis of primary $BH_4$ deficiency were excluded from the study, as were those with concurrent conditions such as seizure disorders, insulin-dependent diabetes mellitus or serious neuropsychiatric illnesses. Other exclusion criteria included alanine aminotransferase more than 5 times the upper limit of normal; a need for concomitant medication with corticosteroids, methotrexate, levodopa, or vaccines during the study; or the use of an investigational agent within 30 days of screening. Pregnant or breastfeeding women or those who were considering pregnancy were excluded, and all sexually active, nonsterile patients agreed to use adequate contraceptive measures during the study.

## Study design

At the screening assessment, patients had their medical and treatment history documented, underwent a physical examination and provided blood for laboratory tests, including analysis of Phe levels. Eligible patients began treatment with sapropterin within 6 weeks of the screening assessment.

Sapropterin dihydrochloride ((6*R*)-2-amino-6-[(1*R*,2*S*)-1,2-dihydroxypropyl]-5,6,7,8-tetrahydro-4(3*H*)-pteridinone dihydrochloride; BioMarin) was administered orally, once daily for 8 days at a dose of 10 mg/kg, dissolved in 115–230 ml water, orange juice or apple juice. The first dose at baseline (day 1) was administered 3–5 h after a meal and all subsequent doses (days 2–8) were taken 5–10 min before breakfast. All patients were instructed to continue their usual dietary habits throughout the study.

Patients were monitored for adverse events (AEs) by a combination of medical interview, physical examination and laboratory tests (biochemistry, haematology, thyroid function tests and urinalysis) at baseline and on days 4, 8 and 36 (±3 days). Safety events were coded according to the Medical Dictionary for Regulatory Activities (MedDRA).

Plasma Phe concentrations in all samples were determined by a central laboratory (Mayo Clinical Trial Services, Biochemical Genetics Laboratory, Department of Laboratory Medicine and Pathology, Rochester, MN, USA) (Grier et al 2004). Phe was measured prior to the first dose of sapropterin on day 1 as a baseline measurement and after completion of the course on day 8. Phe levels were tested 2.5–5 h after a meal and at a similar time of day for both samples.

## Statistical analysis

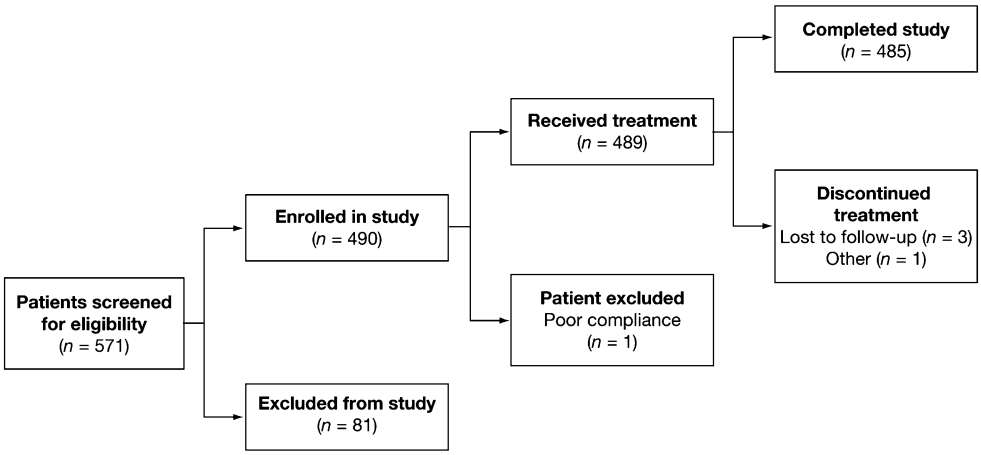
Response to sapropterin was defined as a ≥30% reduction in baseline Phe level on day 8. The choice of the 30% threshold was chosen arbitrarily. The overall percentage of patients who experienced a response on day 8 was calculated and the 95% confidence intervals (CIs) were determined. Additionally, the percentage of patients who experienced a response on day 8 was calculated for each of the baseline Phe level subgroups: <600 μmol/L, 600 to <900 μmol/L, 900 to <1200 μmol/L, and ≥1200 μmol/L. Descriptive statistics for the change from baseline in blood Phe levels were determined for patients who experienced a response on day 8.

All patients who received at least one dose of drug were included in the safety analysis. The safety of treatment was evaluated in an ongoing manner during the study. The sample size was based on the desired precision of the estimated response rates. Before any patients were enrolled in the study, the estimated response rate was 30% and the proposed sample size was 400 patients. Based on these assumptions, the 95%

**Table 1** Demographic characteristics of the 490 patients enrolled

| | |
|---|---|
| Sex, *n* (%) | |
| Male | 236 (48) |
| Female | 254 (52) |
| Age (years) | |
| Mean (SD) | 21.8 (8.9) |
| Age range | 8–48 |
| Race, *n* (%) | |
| Caucasian | 469 (96) |
| Black | 1 (<1) |
| Hispanic | 11 (2) |
| Asian/Pacific Islander | 4 (1) |
| Other | 5 (1) |

**Fig. 1** Patient disposition




CI was 26–35% and this response rate would yield 80–100 patients suitable for enrolment into the subsequent long-term phase III trial. Subsequent calculations showed the observed response rate to be lower than 30%; therefore, the sample size was increased to approximately 700 to maintain 80–100 suitable patients needed for the long-term phase III trial and tighten the precision of the estimated response rate. After the study had enrolled 490 patients, the sponsor determined that 96 patients, sufficient for enrolment in the long-term phase III study, had responded and closed enrolment in this trial.

## Results

A total of 571 patients were considered for enrolment into the study between 4 December 2004 and 7 October 2005. Of these, 490 patients fulfilled the entry criteria and were included in the study. The demographic characteristics of all enrolled patients are shown in Table 1. The ages of the patients in the study ranged from 8 to 48 years, with a mean age of 21.8 years; 87% of patients were aged less than 12 years; 52% of the patients were female and 96% were caucasian.

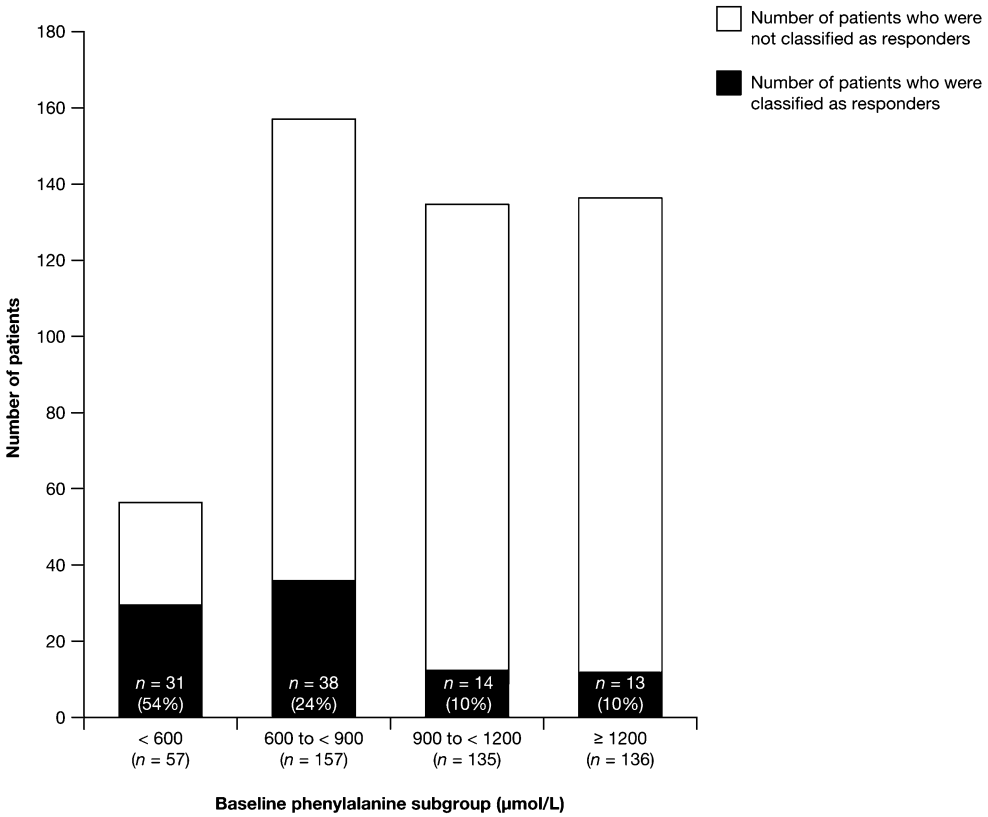



**Fig. 2** Total number of patients and number of patients who responded* to treatment with sapropterin, by baseline phenylalanine (Phe) level subgroup. *A positive response to sapropterin was defined as ≥30% reduction in Phe level between baseline (day 1) and day 8

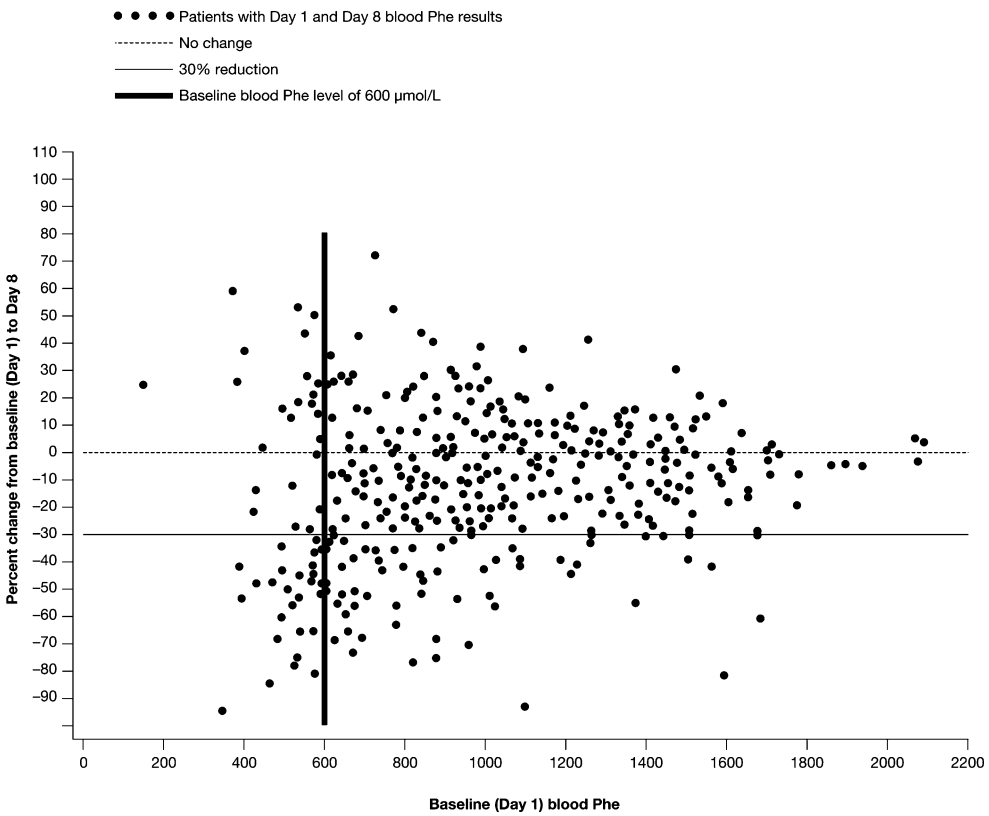



**Fig. 3** Change in phenylalanine (Phe) levels after 8 days of sapropterin by baseline Phe level ($n=495$)

More than 99% (489/490) of patients enrolled received at least one dose of sapropterin. A total of 485 (99%) patients completed the 8-day (±1 day) course of sapropterin and had Phe levels recorded at baseline and on day 8 (Fig. 1). The baseline level was the day-1 Phe level, with the exception of one patient for whom the day-1 Phe level was not available; a Phe value obtained within 4 weeks prior to enrolment as part of the study eligibility evaluation was used instead. Stratification by baseline Phe levels showed that 12% (57/485) of patients had baseline Phe levels <600 µmol/L, 32% (157/485) had baseline Phe levels of 600 to <900 µmol/L, 28% (135/485) had baseline Phe levels of 900 to <1200 µmol/L and 28% (136/485) had baseline Phe levels ≥1200 µmol/L.

## Efficacy

Overall, 20% (96/485; 95% CI 16–23%) of patients with PKU responded to sapropterin (≥30% reduction in blood Phe level) over the 8-day study. Among those who responded, mean (± standard deviation (SD)) Phe levels decreased from day 1 to day 8 by 391.8 (± 185.3) µmol/L.

Response to sapropterin was observed among patients in each of the four baseline Phe level subgroups, including those with baseline Phe levels ≥1200 µmol/L; although the lower baseline Phe level subgroups had higher rates of response to treatment (Fig. 2). In total, 54% (31/57) of patients with baseline Phe levels <600 µmol/L responded to treatment compared with 15% (65/428) of patients with baseline

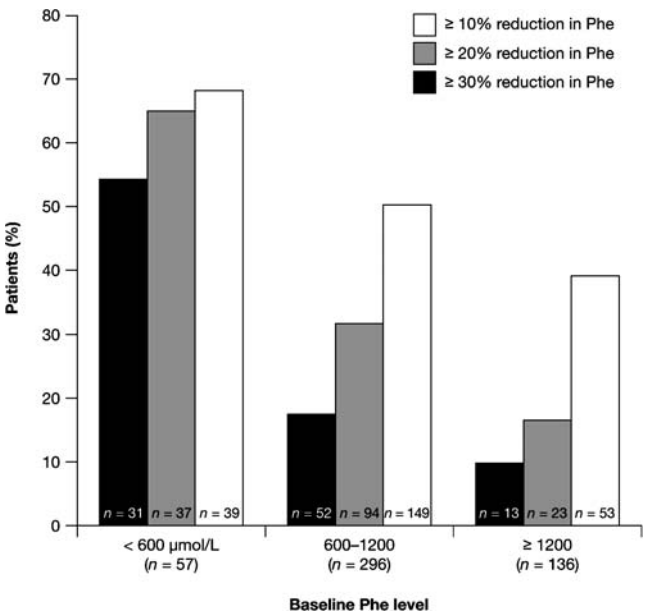



**Fig. 4** Percentage of patients who experienced a reduction in phenylalanine (Phe) of ≥30%, ≥20% and ≥10%, by baseline Phe level ($n=489$)

**Table 2** Adverse events reported by at least 2% of the 489 patients in the safety cohort

| MedDRA organ class | MedDRA-preferred term | Patients *n* (%) |
|---|---|---|
| Gastrointestinal disorders | Diarrhoea | 24 (5) |
| | Abdominal pain | 23 (5) |
| | Nausea | 16 (3) |
| | Flatulence | 11 (2) |
| | Vomiting | 9 (2) |
| Metabolism and nutrition disorders | Decreased appetite | 8 (2) |
| Respiratory, thoracic and mediastinal disorders | Pharyngolaryngeal pain | 9 (2) |
| Infections and infestations | Upper respiratory tract infection | 17 (3) |
| Nervous system disorders | Headache | 50 (10) |
| | Hyperreflexia | 10 (2) |
| | Tremor | 9 (2) |
| General disorders | Fatigue | 14 (3) |

MedDRA = Medical Dictionary for Regulatory Activities.

Phe levels ≥600 μmol/L. However, considerable variability in individual patient response to treatment was observed across all baseline Phe levels (Fig. 3).

Since the definition of a 30% reduction in Phe as a response was chosen arbitrarily, a post hoc analysis was performed to assess the proportion of patients who demonstrated a ≥20% or ≥10% reduction in Phe (Fig. 4). The results for ≥10% reduction must be interpreted with caution owing to assay variability and the natural physiological variability in Phe levels in these patients.

### Safety

A total of 482 AEs were reported in 48% of the 489 patients included in the safety analyses. Only 281 AEs were considered to be possibly or probably treatment related. AEs that occurred in >2% of patients are listed in Table 2. The most common AEs included gastrointestinal disorders, such as abdominal pain and diarrhoea, and minor neurological symptoms, including headache. Most AEs were rated by the investigator as mild to moderate in severity. However, five AEs were rated as severe in 1% (4/489) of patients and included vomiting, headache and migraine, and thrombocytopenia. No patient discontinued the study for an AE.

No deaths occurred during the study. One patient developed appendicitis between the final dose of sapropterin and day 36, but the condition was not considered to be treatment related. No other serious AEs occurred.

## Discussion

During this large phase II screening study, 20% (96/485) of patients with PKU showed a response to sapropterin treatment (with ≥30% reduction in blood Phe from baseline) and were therefore suitable for enrolment into a phase III clinical trial. Furthermore, response to treatment with sapropterin was observed across all baseline subgroups and a substantial number of patients with high baseline Phe levels (≥1200 μmol/L) responded to treatment.

A significant body of evidence indicates that 6*R*-$BH_4$ provides reliable, long-term control of Phe levels in many patients who have HPA as a result of PKU. The findings of this study are consistent with such data. However, the overall response rate to sapropterin in this study appears to be lower than rates reported in other published studies (Bernegger and Blau 2002; Matalon et al 2005; Muntau et al 2002). Comparison of our results with those of other studies is complicated by differences in patient selection criteria, study methodologies, drug dose and formulations, and data interpretation.

The present study was designed as a screening programme rather than a detailed efficacy study, and the lower response rate observed in this study compared with those rates obtained in other published studies may be expected owing to the stringent patient selection criteria. The minimum Phe level of ≥450 μmol/L identified patients who may benefit from improved Phe control (National Institutes of Health Consensus Development Conference Statement 2001). The Phe level of 450 μmol/L for inclusion and the fact that only 12% of patients had baseline Phe levels <600 μmol/L skewed the study population towards patients with higher Phe levels compared with other studies and may have biased against the enrolment of potential responders.

Several smaller studies have reported high response rates to treatment with $BH_4$/6*R*-$BH_4$. One such study

by Muntau and colleagues (2002) involved administration of a single dose of 20 mg/kg of $BH_4$ to 38 patients after dietary Phe loading. The study demonstrated that therapeutic $BH_4$ led to increased PAH activity and accelerated oxidation of Phe. All (10/10) patients with baseline Phe levels <600 μmol/L and 81.0% (17/21) of patients with baseline Phe levels 600 to <1200 μmol/L at screening responded to $BH_4$ (≥30% reduction in Phe levels 15 h after treatment). However, none of the seven patients with baseline Phe levels >1200 μmol/L responded to $BH_4$. Patients in the Muntau and colleagues (2002) study received a different drug formulation and different dosing regimen, after dietary Phe loading, from patients in our study and response rates are therefore not comparable.

Response to sapropterin treatment in our study was observed across all baseline subgroups, although patients with baseline Phe levels <600 μmol/L appeared to have the highest response rates. Many studies have demonstrated response to $BH_4$/6*R*-$BH_4$ in patients with mild to moderate PKU (Cerone et al 2004; Kure et al 1999; Lindner et al 2001; Matalon et al 2005; Muntau et al 2002; Shintaku et al 2003), but few have shown response in patients with classic PKU. Our findings concur with those of a small number of previously published studies that demonstrated a response to 6*R*-$BH_4$ in patients with more severe phenotypes (Fiege et al 2005; Hennermann et al 2005; Matalon et al 2005). A study by Matalon and colleagues (2005) assessed the proportion of patients who responded to a single oral dose of 10 mg/kg of 6*R*-$BH_4$. In total, 58% (22/38) of patients responded to treatment (≥30% reduction in Phe level 24 h after treatment). When analysed by baseline groups, response rates were 67% (2/3) in patients with baseline Phe levels <360 μmol/L; 73% (8/11) in patients with Phe levels 360 to 1200 μmol/L; and 50% (12/24) in patients with Phe levels >1200 μmol/L. Few comparisons can be made with our study owing to differing baseline groups, but the high response rate in patients with baseline Phe levels >1200 μmol/L in the Matalon and colleagues (2005) study indicates that 6*R*-$BH_4$ treatment may also be effective in patients with more severe disease. It must be noted, however, that most patients who had baseline Phe levels >1200 μmol/L in the Matalon and colleagues (2005) study had PAH mutations that corresponded to a functional Phe enzyme in at least one allele. This suggests that they were not patients with true classic PKU and may represent a group with less severe disease than patients with equivalent baseline Phe levels in other studies, including this report. Given the variability of response observed across all phenotypes in this study, it seems prudent to offer a screening test to all patients with PKU regardless of baseline Phe level to evaluate response to sapropterin.

Although a dosage of 10 mg/kg per day has been used in many studies, increased rates of response have been reported in patients receiving higher doses of 6*R*-$BH_4$. Matalon and colleagues (2005) treated 20 patients with 10 mg/kg per day of 6*R*-$BH_4$ for 7 days and then, after a washout period, the same 20 patients were treated with 20 mg/kg per day of 6*R*-$BH_4$ for a further 7 days. The study demonstrated that more patients responded to the higher dose of 6*R*-$BH_4$. Bernegger and Blau (2002) also showed that higher rates of response to 6*R*-$BH_4$ were achieved by loading with a dose of 18–23 mg/kg compared with a dose of <18 mg/kg. These findings suggest that it may be reasonable to monitor the response of each patient to 6*R*-$BH_4$ and adjust the treatment dose accordingly. The 20 mg/kg per day may be a better approach for discrimination between responders and nonresponders, with subsequent adjustment based on the individual requirements of each patient.

In common with the present study, most studies of 6*R*-$BH_4$ in patients with PKU have used a reduction in baseline Phe level of ≥30% as a meaningful response to treatment. However, this 30% threshold is an arbitrary measurement and in clinical practice a lesser reduction in Phe level may represent a clinically meaningful outcome (Fig. 4). Ultimately, the significance of all reductions in Phe levels must be determined in the clinical setting (Matalon et al 2005).

The control of blood Phe is complex and is affected by a wide range of factors, resulting in wide intra-individual variability. In this study, some patients showed changes of up to 70% in blood Phe (Fig. 3). While decreases in blood Phe were largely attributed to sapropterin, the role of intra-individual variability is an important factor to consider when evaluating PKU patients for sensitivity and a control group is clearly desirable for studies in this population. From this study we are not able to determine the positive or negative predictive value of the 8-day administration test to identify patients who respond to sapropterin. However, it is interesting to note that more responders were identified among those with lower baseline blood Phe levels than in those with higher levels, suggesting that at least some of the responses observed were due to sapropterin. This is supported by a randomized, double-blind, placebo-controlled study that demonstrated a significant difference in blood Phe reduction compared with placebo (Levy et al 2006, 2007). Although the current study used a single (day 8) sample to determine response to sapropterin, the

degree of variability means that multiple samples (e.g. 24 h, 3 days, 8 days, and perhaps 14 days) may be advisable to define true responsiveness.

Sapropterin was well tolerated throughout this phase II trial. Most AEs were related to gastrointestinal disorders or minor neurological symptoms, and the majority of AEs were mild and all resolved without complications. The only reported serious AE was not considered to be treatment related.

In conclusion, sapropterin at a dose of 10 mg/kg per day was well tolerated and reduced Phe levels in patients with PKU over this 8-day Phase II, open-label screening study. Despite the stringent study design, response to sapropterin was seen across the spectrum of PKU phenotypes tested, including patients with more severe disease. This study suggests that sapropterin will offer a valuable therapeutic alternative to or addition to dietary control for many patients with PKU. The observed variability in response to sapropterin indicates that response cannot be reliably predicted by baseline Phe levels; a loading test may, therefore, be necessary to detect sapropterin-responsive patients with PKU. Further studies are required to investigate the long-term safety and efficacy of sapropterin.

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