

Sapropterin dihydrochloride for phenylketonuria and tetrahydrobiopterin deficiency

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Sapropterin dihydrochloride is the first registered synthetic form of the naturally occurring cofactor and cosubstrate, tetrahydrobiopterin (BH4). It is essential for the conversion of phenylalanine (Phe) by phenylalanine-4-hydroxylase (PAH) to tyrosine. BH4 is also the co-factor of rate-limiting enzymes involved in the synthesis of monoamine neurotransmitters. Phenylketonuria (PKU) is an inherited disorder of PAH, characterized by elevated Phe concentrations (hyperphenylalaninemia) in the blood and brain, with toxic neurological consequences. Sapropterin dihydrochloride is approved for treating patients (of all ages in the USA and >4 years old in Europe) with PKU who are BH4 responsive, and those with BH4 deficiency (Europe). It decreases blood Phe concentration and increases dietary Phe tolerance in some patients with PKU on a low-Phe diet, allowing dietary adjustment or even discontinuation of a low-Phe diet. This article reviews sapropterin dihydrochloride for the management of PKU – aimed at improving clinical outcomes and quality of life – and it considers the potential for incorporating such information into international consensus guidelines.

KEYWORDS: hyperphenylalaninemia • phenylalanine-4-hydroxylase • phenylketonuria • sapropterin dihydrochloride • tetrahydrobiopterin

Phenylketonuria

Phenylketonuria (PKU; Online Mendelian Inheritance in Man No. 262600) is a genetic disorder characterized by a deficiency of the hepatic enzyme phenylalanine-4-hydroxylase (PAH; EC 1.14.16.1), causing elevated concentrations of phenylalanine (Phe) in the blood and brain. Hyperphenylalaninemia (HPA) can also be caused by a deficiency of tetrahydrobiopterin (BH4), a cofactor of PAH (BH4 deficiency). Phe is an essential amino acid, which is not synthesized *de novo* and must be obtained by diet. PAH converts Phe to tyrosine, an important precursor for catecholamines and melanine [1]. PKU is an inherited, autosomal recessive disease caused by mutations in the *PAH* gene. More than 550 disease-causing mutations have been identified in patients with PKU or HPA [101,102]. The majority of genetic alterations in the *PAH* gene are missense mutations, but splice-site, nonsense and silent mutations, as well as frame shifts and larger deletions and insertions, may also occur. Different mutations affect the activity of the PAH enzyme to different extents, and this may account for the wide variation in blood Phe

concentrations among patients with PKU [2,3]. The location and type of mutations in the *PAH* gene cannot yet be used to fully predict clinical phenotype and, currently, genotyping of the *PAH* gene has limited value in the diagnosis of PKU phenotypes [1].

Almost all cases of PKU in industrialized countries are detected via newborn screening programs, used across Europe since the 1960s. Higher blood Phe concentrations are associated with more severe disease and a greater risk of neurological impairment and, as such, require more urgent treatment. A general classification of PKU disease severity as a function of Phe concentrations is shown in TABLE 1 [4,5]. However, several classifications exist and they vary depending on the practices carried out in individual countries.

The increased circulating concentration of Phe resulting from PKU and HPA is thought to have a neurotoxic effect in the brain [4]. If left untreated from birth, PKU leads to white matter abnormalities [6] and severe mental retardation [7], with losses of cognitive and executive function [8,9]. Although early intervention to

Table 1. Classification of phenylketonuria disease severity according to blood phenylalanine concentrations.

Disease severity	Blood Phe concentrations (range $\mu\text{mol/l}$)
Classic PKU	>1200
Mild–moderate PKU	600–1200
Non-PKU HPA (MHP)	120–600
Normal value in healthy person	<120

HPA: Hyperphenylalaninemia; MHP: Mild hyperphenylalaninemia; Phe: Phenylalanine; PKU: Phenylketonuria. Data from [4,5].

reduce Phe concentrations may avoid serious effects, insufficient control of Phe concentrations may have lasting consequences, such as a lower intelligence quotient, delayed speech, memory deficits, attention problems and behavioral issues [7,10–13]. The consequences of elevated blood Phe concentrations are either long term or transient, and they depend on the age of the patient.

Early-treated adults who discontinue dietary treatment (who are considered ‘off-diet’) are more likely to have vitamin deficiencies and are at risk of neurological and psychological deterioration, giving rise to late-onset epilepsy, ataxia, tremor and problems such as depression, neuroses and anxiety. Women with PKU who are off-diet and become pregnant may develop maternal PKU syndrome, and the outcomes in the newborn child may manifest as facial dysmorphisms, severe mental retardation, microcephaly, developmental delay and congenital heart disease [14].

The prevalence of PKU varies by geographical region and among ethnic groups. The overall prevalence of PKU in Europe is approximately one in 10,000 [15]. The prevalence of PKU in Europe and the Middle East is highest in Northern Ireland and Turkey (one in <5000) [16,17] and in the Gaza strip (one in 3500) [18], but lowest in Finland (one in >100,000) [19].

The main treatment goals for PKU are to maintain blood Phe concentrations within safe limits (e.g., 120–360 $\mu\text{mol/l}$ [Italy], or 120–240 $\mu\text{mol/l}$ for pregnant women), to prevent mental retardation, and to ensure normal growth and normal life with good health throughout adulthood [20]. Guidelines for the treatment of PKU vary between countries and in some cases are quite dated [7,21–24,103]. In general, strict management by dietary control is advocated in early childhood and, although it may become relaxed in older children and those entering adulthood, lifelong management is now recommended to avoid neurological effects.

Until now, the main treatment option in PKU involved putting patients on a restricted, low-Phe diet. Today, however, a new treatment, sapropterin dihydrochloride, is available that may provide good Phe control in some patients, with the possibility of making adjustments towards a more normal diet. The aim of this article is to provide an up-to-date overview of sapropterin dihydrochloride in the management of PKU and BH4 deficiency, and its potential role in international consensus guidelines. The overall objective is to improve clinical outcomes and quality of life for patients with PKU.

Overview of the market

The current treatment for PKU is to decrease Phe concentrations by restricting patients to a low-protein diet that is supplemented with a Phe-free mixture of amino acids, minerals, vitamins and other nutrients [20]. Unfortunately, adherence to current therapy is not always optimal because up to 75% of patients with PKU become essentially nonadherent in adulthood [25,26]. The restrictive low-Phe diet, although effective in preventing most of the adverse effects of HPA on neuropsychological development [4], may lead to nutritional abnormalities, such as deficiencies in iron, tyrosine and fatty acids [27–29], over-reliance on low-quality nutrients (particularly proteins, which may have an impact on metabolism) and abnormal energy intake owing to reduced carbohydrates and fats. The possible clinical effects of these nutritional deficiencies include low bone mass, leading to osteopenia/osteoporosis, growth retardation, weight gain or obesity, and neurocognitive defects [20,28–31]. With the aim of improving clinical outcomes and patient adherence, research has focused on the development of a novel pharmacologic option for PKU. Early studies reported by Kure *et al.* demonstrated reductions in blood Phe concentrations in patients with HPA in response to BH4 [32]. Since then, better BH4 responsiveness has been observed with the less severe variants of PKU [33].

Alternative therapies serving as adjuncts in the management of PKU include more palatable low-Phe foods, such as glycomacropptide [34], as well as large neutral amino acids that compete for and inhibit the uptake of Phe via intestinal absorption or the blood–brain barrier at the LAT1 transporter [35]. Future treatment strategies in PKU focus on enzyme replacement with phenylalanine ammonia lyase (which degrades Phe into nontoxic products) [36], gene delivery [37] and repopulation of hepatic PAH activity by liver cell transplantation [38].

Sapropterin dihydrochloride

Sapropterin dihydrochloride (Kuvan® [BioMarin, CA, USA]) is the first and only registered synthetic form of the naturally occurring enzyme cofactor, BH4. Sapropterin is an orally active, synthetic dihydrochloride salt formulation of the biologically active 6R-diastereoisomer of BH4 (5,6,7,8-tetrahydrobiopterin) [104]. Although PKU is characterized by a defect in the PAH enzyme, residual enzymatic activity may be present in some patients. Thus, sapropterin may act like a chemical chaperone to promote the normal metabolism of Phe and lower its concentration in the blood in a subset of patients who are BH4 responsive. Sapropterin may be used to assist in the control of Phe concentrations. It provides the opportunity for patients who respond to BH4 to adjust their diet, thereby allowing a greater intake of Phe or even coming off of their Phe-restrictive diet. The overall frequency of BH4 responsiveness across Europe is estimated to be 55–62%, based on projections made using genetic allelic data for BH4 responsiveness, although responsiveness can only be determined by a response test [39,40]. Lower rates of BH4 responsiveness are demonstrated with BH4 loading tests (see later) [33,41]. Although the genotype cannot predict BH4 responsiveness with 100% accuracy, it has been documented that residual PAH activity (due to specific mutations) is strongly associated with the BH4-responsive

phenotype and that the presence of two inactive alleles (severe classical PKU) effectively excludes the possibility of BH₄ responsiveness [42]. Sapropterin is also essential for the treatment of HPA in patients of all ages with BH₄ deficiency.

Sapropterin dihydrochloride is available as an oral soluble tablet formulation (100 mg is equivalent to 77 mg of sapropterin) in Europe and in the USA. The formulation contains ascorbic acid, crospovidone, anhydrous calcium hydrogen phosphate, mannitol, riboflavin and sodium stearyl fumarate; ascorbic acid protects the active compound from oxidation [105]. Sapropterin dihydrochloride is also available in Japan as a granule formulation (Biopten[®] granules 2.5%, Asubio Pharma Company Ltd, Tokyo, Japan) [106].

Biochemistry

As mentioned previously, BH₄ is a cofactor and cosubstrate for PAH (FIGURE 1). Mutations in genes encoding for enzymes involved in the biosynthesis or regeneration of BH₄ (GTP cyclohydrolase, 6-pyruvoyl-tetrahydropterin synthase, dihydropteridine reductase [DHPR] and pterin-4a-carbinolamine dehydratase) lead to BH₄ deficiency [43]. The differential diagnosis of PKU versus BH₄ deficiency may be carried out by analyzing blood or urinary pterins and determining blood DHPR activity [44].

Tetrahydrobiopterin is also a cofactor for several enzymes such as tyrosine hydroxylase, tryptophan hydroxylase, glyceryl ether mono-oxygenase and nitric oxide synthase. It is involved in the synthesis of monoamine neurotransmitters, including noradrenaline (norepinephrine), dopamine and 5-hydroxytryptamine

(serotonin), and free radical nitric oxide, and patients with BH₄ deficiencies may display neurological symptoms arising from the impaired production of these neurotransmitters [44].

The rationale for administering sapropterin to patients with BH₄-responsive PKU is to enhance the activity of the defective PAH and thereby increase or restore the oxidative metabolism of Phe sufficient to reduce or maintain blood Phe concentrations, prevent or decrease further Phe accumulation, and increase tolerance to Phe intake in the diet [105].

The mode of action of BH₄ in PKU is not elucidated fully, although several possible mechanisms have been proposed. Additional exogenous BH₄ may promote the activity of mutant low-affinity PAH by increasing BH₄ concentration. Alternatively, BH₄ may act as a chemical chaperone to stabilize mutant proteins and protect them from aggregation, unfolding, proteolytic cleavage and degradation [45–47]. *In vitro* studies of PAH mutants obtained from patients with various phenotypes (including mild HPA and PKU) indicate that BH₄ responsiveness is likely to be multifactorial, stemming from several of these mechanisms [48].

Pharmacodynamics & pharmacokinetics

Preclinical studies

In animal studies, total sapropterin (biopterin) concentrations were approximately 1.5-fold higher in 2-week-old versus 6-week-old rats after oral administration of sapropterin 10 mg/kg, highlighting the potentially higher degree of absorption of sapropterin from the GI tract of younger animals [49].

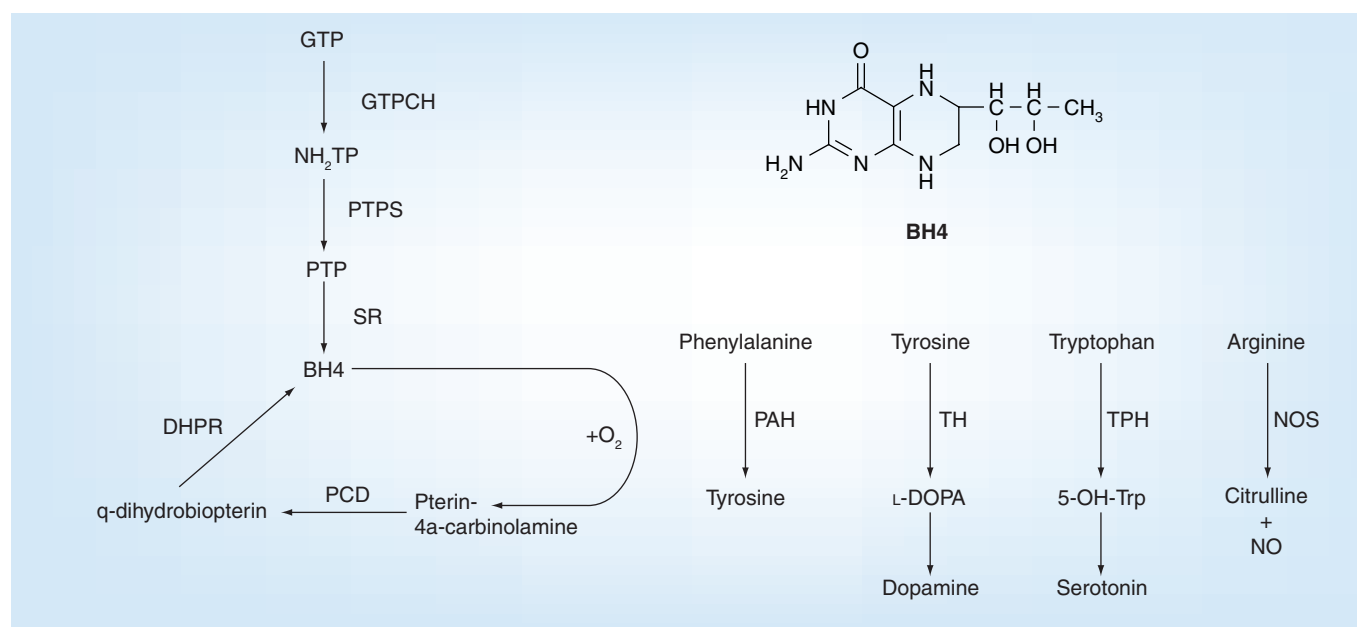


Figure 1. Metabolism and functions of tetrahydrobiopterin (BH₄). Biosynthetic enzymes: GTPCH, PTPS and SR. Regenerating enzymes: PCD and DHPR. BH₄-requiring enzymes: PAH, TH, TPH and NOS. Intermediates in the biosyntheses: NH₂TP and PTP. Metabolites: L-DOPA, 5-OH-Trp and NO.

5-OH-Trp: 5-hydroxytryptophan; BH₄: Tetrahydrobiopterin; DHPR: Dihydropteridine reductase; GTPCH: GTP cyclohydrolase I; L-DOPA: 3,4-dihydroxyphenylalanine; NH₂TP: Dihydroneopterin triphosphate; NO: Nitric oxide; NOS: Nitric oxide synthase; PAH: Phenylalanine-4-hydroxylase; PCD: Pterin-4a-carbinolamine dehydratase; PTP: 6-pyruvoyl-tetrahydropterin; PTPS: 6-pyruvoyl-tetrahydropterin synthase; SR: Sepiapterin reductase; TH: Tyrosine-3-hydroxylase; TPH: Tryptophan-5-hydroxylase.

Biodistribution studies in animal models show that sapropterin, once absorbed, is distributed mainly to the liver, adrenal glands and kidneys [107]. In rats, following intravenous radiolabeled sapropterin administration, radioactivity was detected in fetuses, and the excretion of total sapropterin (biopterin) in milk was demonstrated via the intravenous route. However, no increase in sapropterin concentration in either the fetuses or milk was observed after oral administration (10 mg/kg) [49].

Human studies

Three initial Phase I pharmacokinetic studies, performed in a total of 24 healthy adult subjects using the granule formulation of sapropterin (with doses of 100–200 mg, three-times daily), assessed the safety and tolerability of single- and multiple-dose regimens for up to 7 days of treatment [107]. Absolute bioavailability for humans after oral administration is not known, but in repeat oral-dosing studies there was no accumulation or persistence [107].

In a 12-week, fixed dose, open-label extension study to assess the pharmacokinetics of sapropterin (5, 10 and 20 mg/kg/day) among 78 patients, the best structural model to describe the pharmacokinetic profile of sapropterin was a two-compartment model with first-order input, first-order elimination and a baseline endogenous BH₄-concentration term [50]. Total bodyweight was the only significant covariate identified. The mean (standard deviation [SD]) terminal half-life was 6.69 (2.29) h with little evidence of accumulation, even at the highest dose. The pharmacokinetics of sapropterin (5–20 mg/kg/day) support once-daily dosing.

The pharmacokinetic parameters were estimated after the administration of unregistered BH₄ at 2, 10 and 20 mg/kg to four healthy volunteers [51]. After administration of BH₄ 10 mg/kg, maximal plasma biopterin concentrations peaked between 1 and 4 h, T_{max} (1–4 h), C_{max} (258.7–259.0 nmol/l), the AUC was 1708–1958 nmol·h/l (three subjects) up to 10 h and the elimination half-life was 3.3–5.1 h. Doubling the BH₄ dose to 20 mg/kg/day caused a 60% increase in AUC.

Administration of a single dose of sapropterin (10 mg/kg) leads to a significant lowering of blood Phe concentration within 24 h in most patients with BH₄-responsive PKU, although the maximal effect on Phe concentration may take up to 1 month (depending on the patient and type of mutation) [52]. A single daily dose of sapropterin is sufficient to maintain a stable blood Phe concentration over a 24-h period, with no substantial increase in Phe concentration observed after food intake [41,53].

An open-label, three-treatment, six-sequence, three-period crossover, Phase I trial (PKU013) [54] involving healthy volunteers (n = 32) showed that the geometric mean ratio of the AUC from baseline to time 't' ($AUC_{(0-t)}$) for intact versus dissolved tablets, under fasting conditions, was 141.24% (90% CI: 122.05–163.43); for intact tablets, under-fed versus fasting conditions, $AUC_{(0-t)}$ was 143.46% (90% CI: 124.22–165.69). Oral administration of sapropterin as intact tablets with a high-fat, high-calorie meal was associated with increased drug exposure. It is recommended that sapropterin is administered after dissolution in water, with a meal, at the same time each day, preferably in the morning.

Metabolism

Sapropterin is primarily metabolized in the liver to dihydrobiopterin and biopterin, and to some extent is converted non-enzymatically to pterin, probably by side-chain cleavage. It has been shown that only a small portion of orally administered BH₄ is excreted in urine as biopterin or converted to lumazines in the gut [55,56]. It is hypothesized that most of the ingested BH₄ is used as a cofactor (mainly for PAH in the liver) and catabolized to nonfluorescing compounds; it is possibly then degraded to CO₂ and ammonia. After intravenous injection of low-dose [¹⁴C]BH₄ (45 µg/kg) in mice, high levels of radioactivity were detected in the liver and kidney; very little was found in the brain, adrenal medulla and bone marrow [57]. As sapropterin is a synthetic version of the naturally occurring active isomer of BH₄ (6R-BH₄), it can be expected to undergo the same metabolism [105]. Repeated administration of sapropterin did not upregulate cytochrome P450 (CYP)-dependent drug-metabolizing enzymes in the liver microsomes [41]. Rat excretion studies have shown that 72 h after the oral administration of 100 mg/kg of radioactive sapropterin, 75% was excreted in feces and 7% in urine [49].

Clinical efficacy: Phase II & III studies

PKU-001 was a Phase II screening study to evaluate the response to an 8-day course of sapropterin (10 mg/kg/day) in 490 patients with poorly controlled PKU of varying severity (TABLE 2) [41]. A response to sapropterin (decrease in blood Phe of ≥30%) was observed in 20% of patients overall, with a higher response rate in subgroups with lower baseline blood Phe (54, 24, 10 and 10% for baseline blood Phe <600, 600–900, 900–1200 and ≥1200 µmol/l, respectively). Sapropterin was well tolerated.

In a Japanese study, BH₄-responsive patients with HPA administered in a single BH₄ dose of 10 mg/kg responded similarly to patients given the higher BH₄ dose of 20 mg/kg (as favored in Europe) [52]. A dose-dependent response to BH₄ administration, in terms of the measured reduction in blood Phe concentration, as described elsewhere in a genetically homogenous PKU population [58], was clearly evident.

A further Phase II study was designed to investigate the safety of sapropterin in the management of blood Phe concentrations in patients with primary BH₄ deficiency (PKU-007) (TABLE 2) [59]. A total of 12 patients received sapropterin (up to 20 mg/kg/day) for 10 weeks, followed by a 7-week extension period. An interim analysis demonstrated a favorable safety profile in these patients, and most patients maintained blood Phe concentrations within an acceptable range for up to 10 weeks of treatment (<360 µmol/l).

PKU-003 recruited responders to sapropterin (≥30% reduction in blood Phe on sapropterin) from the earlier Phase II study (PKU-001, see above) [53]. Patients were randomized to double-blind treatment with sapropterin or placebo for 6 weeks. Blood Phe in the sapropterin group was reduced within the first week, and remained low throughout the remainder of the study (mean [SD] changes vs baseline in blood Phe concentrations at 6 weeks were -236 [257] µmol/l compared with +3 [240] µmol/l

for placebo [$p < 0.0001$]). However, only 44% of the responders from the PKU-001 study were true responders (vs 9% on placebo). The low number of responders in the PKU-003 study may be due to the fact that in PKU-001, the responsiveness was tested at home, and in some nonresponder patients, the protein intake was not constant, thus producing false-positive results.

A total of 80 patients from PKU-003 were enrolled in PKU-004, a 22-week open-label extension (TABLE 2) [60]. This was a 6-week forced-titration phase (consecutive 2-week periods of 5, 20 and 10 mg/kg/day), a 4-week dose-analysis phase (at 10 mg/kg/day) and a 12-week fixed-dose phase (dosage based upon their blood Phe response during dose titration. Almost half of patients (46%) responded to sapropterin.

The other pivotal Phase III study (PKU-006 [TABLE 2]) investigated the capability of sapropterin to increase Phe tolerance while maintaining adequate blood Phe control in children with PKU [61]. In total, 50 out of 89 patients who had their blood Phe measured at day 8 were responsive to sapropterin 20 mg/kg/day ($\geq 30\%$ decrease in blood Phe and blood Phe ≤ 300 $\mu\text{mol/l}$ at day 8), and blood Phe was reduced in these patients by 64% (SD: 17.5%) on average. In the second part of the study, patients in the sapropterin group tolerated significantly more additional dietary Phe while maintaining adequate blood Phe control, consistent with improved Phe tolerance.

Following completion of the previous studies (PKU-004 and PKU-006), patients were given the option of continuing to receive sapropterin in a long-term, open-label safety Phase IIIb trial (PKU-008) (TABLE 2) [62]. Of 111 patients enrolled, 110 received sapropterin (5–20 mg/kg/day) and blood Phe concentrations were maintained in accordance with NIH consensus guidelines [7].

Post-marketing surveillance studies

The Kuvan Adult Maternal and Pediatric European Registry (KAMPER) is an observational, multicenter, multinational drug registry study designed to collect information on the long-term outcomes of patients with HPA (owing to PAH or BH₄ deficiencies) treated with sapropterin [63]. KAMPER was launched in December 2009 (first patient enrollment) and will be ongoing for 15 years. Data will be collected from 625 BH₄-responsive patients in approximately 100 centers in 11 EU countries, and the primary objective is to assess the long-term safety in patients treated with sapropterin. Secondary objectives are to provide additional information regarding safety in specific subgroups, including children aged 4 years or older, pregnant women, the elderly (≥ 65 years old), and patients with renal or hepatic insufficiency. Growth and neurocognitive outcomes in the pediatric population, adherence to diet and to sapropterin treatment, and the long-term efficacy of sapropterin will also be assessed. Interim analyses will be performed annually [63].

In the USA, a similar registry, the PKU Demographic, Outcomes and Safety registry (NCT00778206), was launched in September 2008 [64]. The aim is to enroll up to 3500 patients diagnosed with PKU and HPA and to follow up these patients for up to 15 years. The Maternal PKU Observational Program subregistry

will obtain plasma and breast-milk samples from mothers enrolled in the PKU Demographic, Outcomes and Safety registry who are breastfeeding when their infants are 1-month old [64].

Safety & tolerability

Sapropterin is well tolerated with a favorable risk–benefit profile. The clinical trials program of sapropterin covers a range of doses (5, 10 and 20 mg/kg/day) and durations of therapy. Of the 579 patients who received treatment with sapropterin in clinical trials, approximately 35% experienced adverse reactions. The most commonly reported adverse events (AEs) included headache and rhinorrhea ($\geq 10\%$ of patients). Other AEs that occurred in 4–10% of patients were: pharyngolaryngeal pain, diarrhea, vomiting, cough, abdominal pain, rash, nasal congestion and hypophenylalaninemia [104]. Mild-to-moderate neutropenia was noted during sapropterin administration in 24 out of 579 patients (4%) [104]. In one pivotal Phase III trial (PKU-003), sapropterin was associated with a safety profile similar to that of placebo ($n = 89$); after 6 weeks of treatment, no significant difference between sapropterin and placebo in the frequencies of AEs possibly related to treatment were reported (23 vs 20%, respectively; $p = 0.80$) [53]. In the 22-week extension study (PKU-004), 79 of the 80 patients enrolled completed the study; a total of 32% of patients experienced an AE that was considered possibly or probably related to sapropterin (5–20 mg/kg/day), but no patient withdrew because of an AE [60].

There is no evidence of any safety issues following the long-term administration of sapropterin. Interim analysis of the Phase IIIb extension study (PKU-008) found that, after 18 months, while 71% of subjects reported AEs (79 out of 111), the majority of those reported were mild and not dose dependent [61]. The most common AEs reported were cough (16.2%), pyrexia (14.4%) and nasopharyngitis (13.5%). Only one serious AE (gastroesophageal reflux) was noted, which was thought to be related to sapropterin.

Regulatory affairs

Kuvan received orphan drug designation from the US FDA and from the European Commission in 2004. Regulatory approval was achieved for sapropterin in Europe and the USA based upon the positive outcomes from four clinical trials (PKU-001, -003, -004 and -006) and the accumulating clinical experience attesting to the efficacy and safety of sapropterin in 579 patients with PKU.

Marketing authorization of sapropterin dihydrochloride, in tablet formulation, as therapy for patients with HPA owing to PKU, was granted by the FDA in 2007 (marketed by BioMarin Pharmaceutical Inc. [CA, USA] in the USA and Canada) [104]. In Japan, sapropterin dihydrochloride (Biopten), a granule formulation approved for BH₄ deficiency in 1992, was also approved in 2008 for the treatment of BH₄-responsive patients with HPA (including PKU) [65]. In Europe, sapropterin dihydrochloride received marketing authorization in December 2008 (marketed by Merck Serono SA [Geneva, Switzerland] in Europe and the rest of the world) and it is indicated for the treatment of HPA in adult and pediatric patients 4 years or

Table 2. Summary of Phase II and III clinical trials of sapropterin.

Study	Study type and design	Study number	Summary of objectives	Sapropterin dosage and regimen	Subjects/patients [†]	Key findings	NCT number	Ref.
Burton <i>et al.</i> (2007)	Phase II, multicenter, open-label, uncontrolled screening study	PKU-001	Assess response to an 8-day course of sapropterin in patients with PKU	Sapropterin 10 mg/kg/day, 8 days	Patients (n = 490) were aged 8–49 years; Phe concentration >450 µmol/l; not on Phe-restricted diet	A ≥30% reduction in Phe concentration in 20% of patients (mean Phe was reduced by 392 µmol/l [SD: 185] in these patients)	NCT00104260	[41]
Wasserstein <i>et al.</i> (2008)	Phase II, open-label, multicenter, uncontrolled study	PKU-007	Assess safety and efficacy of sapropterin in patients with primary BH4 deficiency	Sapropterin 5–20 mg/kg/day, 10 weeks (followed by a 7-week extension period)	Patients (n = 12) had primary BH4 deficiency and a history of Phe concentrations >180 µmol/l; diet remained unchanged	Mean Phe concentrations were maintained within an acceptable range (<360 µmol/l) in most patients and sapropterin was well tolerated	NCT00355264	[59]
Levy <i>et al.</i> (2007)	Phase III, randomized, multicenter, double-blind, placebo-controlled study	PKU-003	Evaluate the efficacy of sapropterin to reduce blood Phe concentration	Sapropterin 10 mg/kg/day, 6 weeks	Patients (enrolled, n = 89; safety/ITT, n = 88) were responders [‡] from study PKU-001; patients were not on a Phe-restricted diet	Sapropterin reduced Phe concentration by 236 µmol/l (SD: 257) versus placebo (p < 0.0001); 44% on sapropterin versus 9% on placebo demonstrated a reduction in Phe of ≥30%; a significant difference was evident at week 1 and persisted for the duration of the trial (p < 0.001)	NCT00104247	[53]
Lee <i>et al.</i> (2008)	Phase III, open-label, uncontrolled extension study	PKU-004	Assess the safety and efficacy of sapropterin in the treatment of PKU	Sapropterin, 5, 10, 20 mg/kg/day, 22 weeks	Patients (n = 80) were aged ≥8 years and their diet remained unchanged	Almost half (46%) of patients demonstrated a reduction in Phe concentration of ≥30%; dose-dependent decreases in blood Phe concentration were maintained up to 22 weeks; mean Phe was 652 µmol/l (SD: 382) at week 22; sapropterin was well tolerated; all AEs except one were mild-to-moderate and no AE led to treatment discontinuation	NCT00225615	[60]

[†]A total of 89 had blood Phe measured at day 8.

[‡]Defined as ≥30% reduction in blood Phe concentration from baseline. For PKU-006, responders additionally had blood Phe less than or equal to 300 µmol/l.

AE: Adverse event; BH4: Tetrahydrobiopterin; ITT: Intention-to-treat; Phe: Phenylalanine; PK: Pharmacokinetic; PKU: Phenylketonuria; SD: Standard deviation.

NCT reference refers to the study entry on www.clinicaltrials.gov. Adapted from [1,107].

Table 2. Summary of Phase II and III clinical trials of sapropterin.

Study	Study type and design	Study number	Summary of objectives	Sapropterin dosage and regimen	Subjects/patients ^a	Key findings	NCT number	Ref.
Trefz <i>et al.</i> (2009)	Phase III, open-label, uncontrolled screening study	PKU-006 Part 1	Evaluate sapropterin treatment for increasing Phe tolerance while maintaining adequate blood Phe control in children with PKU	Sapropterin 20 mg/kg/day, 8 days	Patients (n = 90) enrolled were aged 4–12 years; Phe concentration <480 µmol/l; patients were on a Phe-restricted diet	Mean Phe reduced from 317 µmol/l (SD: 173) to 108 µmol/l (SD: 70); mean reduction in Phe from day 1 to day 8 was 209 µmol/l (SD: 139) and mean percentage change in Phe was 64% (SD: 17.5)	NCT00272792	[61]
Trefz <i>et al.</i> (2009)	Phase III, randomized, double-blind, placebo-controlled study	PKU-006 Part 2	As above	Sapropterin 20 mg/kg/day, 10 weeks	Patients (n = 46) were responders ^b from Part 1 of study PKU-006 (above); patients were on a Phe-restricted diet	Patients receiving sapropterin tolerated mean additional Phe diet supplementation of 21 mg/kg/day (SD: 2.3) versus 3.3 mg/kg/day (SD: 3.9; p < 0.001) in the placebo group over the 10 weeks, while maintaining Phe <360 µmol/l	NCT00272792	[61]
Fernhoff <i>et al.</i> (2009)	Phase IIb, long-term open-label, uncontrolled extension study	PKU-008	Evaluate the safety of treatment with sapropterin	Sapropterin 5, 10 and 20 mg/kg/day, 3-year study with interim reporting at 18 months	Patients (n = 111) enrolled were responders ^b from the PKU-004 and PKU-006 studies and 110 had received a daily dose of sapropterin; diet remained unchanged	Interim 18-month report: Phe concentration levels were maintained for 18 months between 485 µmol/l (SD: 309) and 530 µmol/l (SD: 332); safety: most AEs were mild and not dose dependent; the most common AEs were cough (16.2%), pyrexia (14.4%) and nasopharyngitis (13.5%)	NCT00332189	[62]

^aA total of 89 had blood Phe measured at day 8.

^bDefined as ≥30% reduction in blood Phe concentration from baseline. For PKU-006, responders additionally had blood Phe less than or equal to 300 µmol/l.

AE: Adverse event; BH4: Tetrahydrobiopterin; ITT: Intention-to-treat; Phe: Phenylalanine; PK: Pharmacokinetic; PKU: Phenylketonuria; SD: Standard deviation.

NCT reference refers to the study entry on www.clinicaltrials.gov.

Adapted from [1,107].

older with PKU who are responsive to BH₄ treatment, and in BH₄-responsive patients with BH₄ deficiency [105]. Under the centralized procedure of the EMA, marketing authorization of sapropterin is valid in all EU states, Iceland, Liechtenstein and Norway, and it was approved in 2009 under separate legislation in Switzerland.

Conclusion

The therapeutic response to sapropterin is clearly demonstrated in clinical studies involving BH₄-responsive patients with PKU. These studies show that sapropterin is effective in this subset of

patients by optimizing Phe management and lowering blood Phe concentrations. Sapropterin-responsive patients may increase the amount of Phe-containing foods in their diet as a result of increased Phe tolerance. A minority of patients may be able to discontinue their low-Phe diet. Consequently, sapropterin may provide the opportunity to overcome nonadherence to a low-Phe diet and the rise in blood Phe concentration that ensues in patients who respond to BH₄. The target group of patients who may benefit from sapropterin administration usually have mild-to-moderate PKU (blood Phe of 600–1200 μmol/l); among patients with classical PKU (blood Phe >1200 μmol/l),

up to 10% of patients may respond [41]. Recommendations for treatment of mild HPA (blood Phe of 120–600 μmol/l) differ between countries; however, the majority of these patients are found to be BH₄ responders and can be potentially treated with sapropterin [4,33]. Responders to BH₄ can be divided into two groups: those who need a combination of sapropterin and a relaxed low-Phe diet, and those who can discontinue the diet completely during treatment with sapropterin. Thus, sapropterin may offer the possibility to reduce the level of nutritional deficiencies that accompany a restricted diet and, potentially, minimize the suboptimal outcomes, such as neurological and psychological dysfunctions. The therapeutic management of BH₄-responsive PKU and BH₄ deficiency using sapropterin may also allow patients to enjoy greater variety in their diets, with fewer limitations in social settings involving food, and to experience improvement in daily quality of life. It is, however, essential to monitor blood Phe levels regularly during sapropterin treatment, in order to ensure that they remain within the recommended therapeutic range.

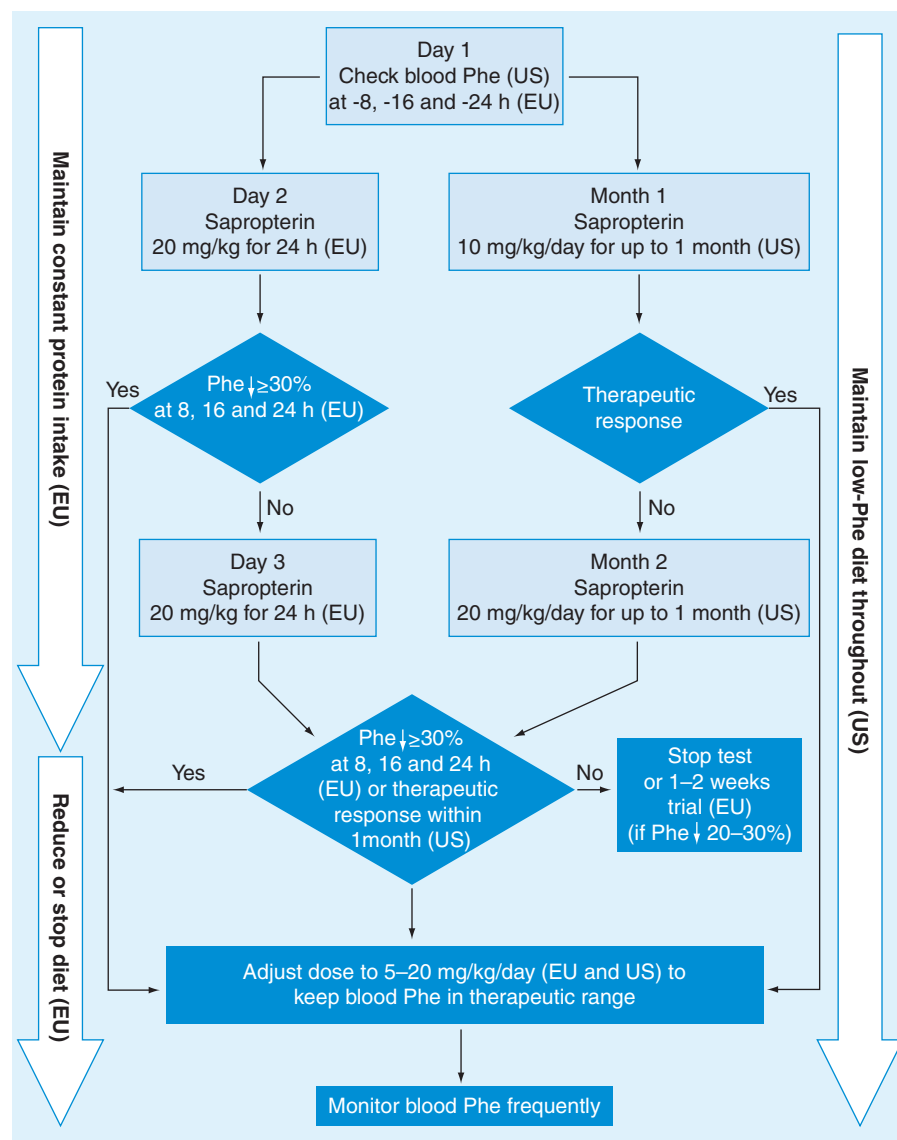


Figure 2. Algorithms for screening and initiating treatment in phenylketonuria patients with sapropterin dihydrochloride based on US Prescribing Information [103] or a recommendation from a group of European experts [1]. These recommendations differ from those in the European Summary of Product Characteristics for sapropterin; according to US Prescribing Information, blood Phe should be checked at 1 week and periodically thereafter for the remainder of the 1-month treatment period; no formal definition for a therapeutic response to sapropterin is provided. Phe: Phenylalanine.

Expert commentary & five-year view

Sapropterin is the first pharmacologic treatment for patients with PKU that has been shown in randomized double-blind trials to be effective in lowering blood Phe concentration and increasing Phe tolerance. Sapropterin is a synthetic formulation of 6R-BH₄. The formulation of sapropterin as oral soluble tablets offers improved stability at room temperature over the earlier, unregistered and unlicensed, tetrahydrobiopterin dihydrochloride tablets. Thus, sapropterin provides a

promising new treatment option for patients with PKU or BH4 deficiency who are also BH4 responsive.

A recent survey of the management of PKU, conducted across 93 treatment centers in 19 countries within Europe, identified that only the minority (34%) of healthcare professionals currently use BH4 as a treatment option in their practice, although approximately half (54%) of the respondents would perform a BH4-responsiveness loading test [66]. It is recommended that all patients with PKU should undergo a BH4 oral-response test before treatment initiation [67]. In Europe, the BH4 loading test is mostly performed in the neonatal period, when Phe concentrations are high and it is practical to perform the test [1]. A 30% or greater reduction in the blood Phe concentration is most widely considered to represent a clinically significant response to treatment; however, this threshold is arbitrary and a lower degree of response might also be considered clinically significant in some individuals [68]. Identification of a simple and universal loading test would facilitate the identification of responders to sapropterin [1].

An optimized algorithm (FIGURE 2) is proposed based on suggested approaches for initiating, and determining response to, therapy with sapropterin from the USA and the EU [104,105]. In the approved Kuvan protocol from the USA, response to sapropterin therapy initiated at 10 mg/kg/day is determined by measuring Phe concentrations after the first week and periodically for up to 1 month [104]. If no therapeutic response is obtained at 10 mg/kg/day, the dose can be increased to 20 mg/kg/day and Phe concentrations measured periodically for a further month. Patients who do not respond to 1 month of treatment at 20 mg/kg/day are deemed to be nonresponders and treatment should be withdrawn. Another proposed European protocol,

also shown in FIGURE 2, closely follows current practice in Europe (and was proposed by an expert group of European physicians) and allows determination of response to sapropterin within 48 h by repeated blood Phe concentration testing, followed by dose adjustment and ongoing, repeated, blood Phe concentration monitoring as part of long-term follow-up [1]. It should be noted, however, that this algorithm differs from that recommended in the European Summary of Product Characteristics for sapropterin [105]. Finally, the use of a combined Phe (100 mg/kg)-BH4 (20 mg/kg) loading test is not recommended (with the exception of the Phe breath test [69]) and may result in apparent nonresponsiveness in both responders and nonresponders to sapropterin [70].

National guidelines for the management of PKU agree that treatment should start as early as possible after birth, and that monitoring Phe concentrations and clinical parameters should continue throughout life [7,21–24,103]. However, the European PKU survey identified great discrepancies in the screening, treatment, follow-up and organization of PKU management between and within European centers [66]. Consequently, there is a need to generate evidence-based international guidelines to optimize the management of PKU and HPA, and to incorporate recommendations for treatment with sapropterin to improve patient outcomes among those who are BH4 responsive [66].

Guidelines for the treatment of HPA caused by PKU were published largely before the availability of sapropterin. Within the context of updating guidelines, sapropterin might be accommodated into current treatment practice in a number of possible scenarios: as an adjunct to the standard dietary practice, allowing relaxation of the low-Phe diet (as long as Phe and tyrosine concentrations remain within recommended limits), or as a full substitute for diet. In addition, the advent of a portable Phe

Key issues

- Phenylketonuria (PKU) is an autosomal recessive disorder that occurs in approximately one in 10,000 to one in 20,000 of the population in Europe and the USA.
- If left untreated from birth, PKU leads to high phenylalanine (Phe) blood concentrations that can result in neurotoxic effects, causing severe mental retardation, brain structure damage, seizures and disturbance in neurotransmitter synthesis.
- PKU therapy has focused firmly on the restriction of dietary Phe intake and excludes many naturally high-protein foods, such as dairy products, meat and fish. As a consequence, supplementation with Phe-free protein substitutes (consisting of essential and nonessential amino acids) is usually required.
- The Phe-restricted diet imposes a substantial burden on the individual with PKU and his or her family by restricting their freedom in life. Typically, only one in four adults with PKU continue to adhere to a low-Phe diet. Despite this, it is increasingly recognized that the management of PKU should be lifelong in order to avoid a range of adverse neuropsychologic symptoms in adulthood.
- Sapropterin is the first registered medication to offer the potential for pharmacologic management of tetrahydrobiopterin (BH4)-responsive patients with PKU or BH4 deficiency, in an easy-to-use formulation.
- The adoption of sapropterin into routine clinical management offers the potential for better Phe control in patients who respond to BH4, increasing Phe tolerance while maintaining target Phe concentrations, and enabling the freedom of a less restrictive diet.
- National guidelines for the management of PKU vary between countries (and even within countries) with respect to recommended blood Phe concentrations during dietary treatment, treatment duration and recommended frequency of monitoring blood Phe concentrations.
- There is an emerging need for evidence-based international guidelines to provide consensus concerning treatment initiation and determination of the response to therapy with sapropterin, as well as consensus on target blood Phe concentrations and clarity on optimized treatment protocols for the management of PKU and hyperphenylalaninemia.
- In the next 5 years, the advances in understanding the disease process of hyperphenylalaninemia and PKU, the wider adoption of sapropterin and the development of international consensus guidelines should lead to the improved management of PKU and, thus, offer many patients better disease outcomes and improved engagement in normal daily life.

monitoring device may motivate patients to adhere more closely to dietary recommendations in efforts to maintain lower Phe concentrations [108].

Over the next 5 years, with the advent of sapropterin, the development of new international guidelines and the adoption of a lifelong approach to the management of PKU, there is now the potential for better disease management in terms of controlling and maintaining target blood Phe concentrations, with the additional possibility of relaxing dietary Phe restrictions in patients who respond to sapropterin. This will allow greater patient freedom with regard to choice of diet, and may avoid many of the clinical consequences resulting from poor dietary control of Phe concentration and nutritional deficiencies, thereby improving clinical outcomes and the quality of life for many patients with PKU.

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Information resources

- Tetrahydrobiopterin
www.bh4.org
- BIOPKU. Databases of pediatric neurotransmitter disorders
www.biopku.org
- ClinicalTrials.gov
www.clinicaltrials.gov
- European Medicines Agency
www.ema.europa.eu
- Food and Drug Administration
www.fda.gov
(No older than 5 years; 2004–2009 plus early 2010)

References

Papers of special note have been highlighted as:

- of interest
 - of considerable interest
- 1 Blau N, Belanger-Quintana A, Demirkol M *et al.* Optimizing the use of sapropterin (BH(4)) in the management of phenylketonuria. *Mol. Genet. Metab.* 96(4), 158–163 (2009).
 - **Important review identifying an optimized treatment protocol for sapropterin therapy.**
 - 2 Scriver CR. The *PAH* gene, phenylketonuria, and a paradigm shift. *Hum. Mutat.* 28(9), 831–845 (2007).
 - 3 Guttler F, Guldberg P. Mutations in the phenylalanine hydroxylase gene: genetic determinants for the phenotypic variability of hyperphenylalaninemia. *Acta Paediatr. Suppl.* 407, 49–56 (1994).
 - 4 Feillet F, van Spronsen FJ, Macdonald A *et al.* Challenges and pitfalls in the management of phenylketonuria. *Pediatrics* (2010) (In press).
 - **Important review presenting the current knowledge in treatment of phenylketonuria (PKU).**
 - 5 Lindner M. Treatment of phenylketonuria variants: European recommendations. In: *PKU and BH4: Advances in Phenylketonuria and Tetrahydrobiopterin*. Blau N (Ed.). SPS Verlagsgesellschaft mbH, Heilbronn, Germany 180–187 (2006).

- 6 Huttenlocher PR. The neuropathology of phenylketonuria: human and animal studies. *Eur. J. Pediatr.* 159(Suppl. 2), S102–S106 (2000).
- 7 NIH. National Institutes of Health Consensus Development Conference Statement: phenylketonuria: screening and management, October 16–18, 2000. *Pediatrics* 108(4), 972–982 (2001).
- **Comprehensive guidelines for PKU management.**
- 8 Hegge KA, Horning KK, Peitz GJ, Hegge K. Sapropterin: a new therapeutic agent for phenylketonuria. *Ann. Pharmacother.* 43(9), 1466–1473 (2009).
- **Includes summary of clinical trials of sapropterin.**
- 9 Scriver CR, Kaufman S. Hyperphenylalanemia: phenylalanine hydroxylase deficiency. In: *The Metabolic and Molecular Bases of Inherited Disease*. Scriver CR, Beaudet AL, Sly WS, Valle D (Eds). McGraw-Hill, NY, USA 1667–1709 (2001).
- 10 Arnold GL, Vladutiu CJ, Orlowski CC, Blakely EM, DeLuca J. Prevalence of stimulant use for attentional dysfunction in children with phenylketonuria. *J. Inher. Metab. Dis.* 27(2), 131–143 (2004).
- 11 Gassio R, Fuste E, Lopez-Sala A, Artuch R, Vilaseca MA, Campistol J. School performance in early and continuously treated phenylketonuria. *Pediatr. Neurol.* 33(4), 267–271 (2005).
- 12 Waisbren SE, Noel K, Fahrbach K *et al.* Phenylalanine blood levels and clinical outcomes in phenylketonuria: a systematic literature review and meta-analysis. *Mol. Genet. Metab.* 92(1–2), 63–70 (2007).
- 13 White DA, Tabor Connor L, Nardos B *et al.* Age-related decline in the microstructural integrity of white matter in children with early- and continuously-treated PKU: a DTI study of the corpus callosum. *Mol. Genet. Metab.* 99(Suppl. 1), S41–S46 (2010).
- 14 Hoeks MP, den Heijer M, Janssen MC. Adult issues in phenylketonuria. *Neth. J. Med.* 67(1), 2–7 (2009).
- 15 Loeber JG. Neonatal screening in Europe; the situation in 2004. *J. Inher. Metab. Dis.* 30(4), 430–438 (2007).
- 16 O'Neill CA, Eisensmith RC, Croke DT, Naughten ER, Cahalane SF, Woo SL. Molecular analysis of PKU in Ireland. *Acta Paediatr. Suppl.* 407, 41–44 (1994).
- 17 Ozalp I, Coskun T, Tokatli A *et al.* Newborn PKU screening in Turkey: at present and organization for future. *Turk. J. Pediatr.* 41(2), 97–101 (2001).
- 18 Abu Shahla AN, Abed Y, Abu Shahla NK. Screening programme for phenylketonuria in the Gaza Strip: evaluation and recommendations. *J. Trop. Pediatr.* 50(2), 101–105 (2004).
- 19 Guldberg P, Henriksen KF, Sipila I, Guttler F, de la Chapelle A. Phenylketonuria in a low incidence

- population: molecular characterisation of mutations in Finland. *J. Med. Genet.* 32(12), 976–978 (1995).
- 20 Giovannini M, Verduci E, Salvatici E, Fiori L, Riva E. Phenylketonuria: dietary and therapeutic challenges. *J. Inherit. Metab. Dis.* 30(2), 145–152 (2007).
- **Review of optimal dietary management of PKU and nutritional challenges.**
- 21 Abadie V, Berthelot J, Feillet F *et al.* Management of phenylketonuria and hyperphenylalaninemia: the French guidelines. *Arch. Pediatr.* 12(5), 594–601 (2005).
- 22 Bremer H, Buhrdel P, Burgard P *et al.* Therapie von Patienten mit Phenylketonurie. Empfehlungen der Arbeitsgemeinschaft für pädiatrische Stoffwechselstörungen (in German). *Monatsschr. Kinderheilkd.* 145, 961–962 (1997).
- 23 Burgard P, Bremer HJ, Buhrdel P *et al.* Rationale for the German recommendations for phenylalanine level control in phenylketonuria 1997. *Eur. J. Pediatr.* 158(1), 46–54 (1999).
- 24 UK MRC. Recommendations on the dietary management of phenylketonuria. Report of Medical Research Council Working Party on Phenylketonuria. *Arch. Dis. Child.* 68, 426–427 (1993).
- **Primary recommendations upon which most other national guidelines are based.**
- 25 Walter JH, White FJ, Hall SK *et al.* How practical are recommendations for dietary control in phenylketonuria? *Lancet* 360(9326), 55–57 (2002).
- 26 Koch R, Burton B, Hoganson G *et al.* Phenylketonuria in adulthood: a collaborative study. *J. Inherit. Metab. Dis.* 25(5), 333–346 (2002).
- 27 Acosta PB, Yannicelli S, Singh R, Elsas LJ, Mofidi S, Steiner RD. Iron status of children with phenylketonuria undergoing nutrition therapy assessed by transferrin receptors. *Genet. Med.* 6(2), 96–101 (2004).
- 28 Koletzko B, Sauerwald T, Demmelmair H *et al.* Dietary long-chain polyunsaturated fatty acid supplementation in infants with phenylketonuria: a randomized controlled trial. *J. Inherit. Metab. Dis.* 30(3), 326–332 (2007).
- 29 Przyrembel H, Bremer HJ. Nutrition, physical growth, and bone density in treated phenylketonuria. *Eur. J. Pediatr.* 159(Suppl. 2), S129–S135 (2000).
- 30 Acosta PB, Yannicelli S, Singh R *et al.* Nutrient intakes and physical growth of children with phenylketonuria undergoing nutrition therapy. *J. Am. Diet. Assoc.* 103(9), 1167–1173 (2003).
- 31 Modan-Moses D, Vered I, Schwartz G *et al.* Peak bone mass in patients with phenylketonuria. *J. Inherit. Metab. Dis.* 30(2), 202–208 (2007).
- 32 Kure S, Hou DC, Ohura T *et al.* Tetrahydrobiopterin-responsive phenylalanine hydroxylase deficiency. *J. Pediatr.* 135(3), 375–378 (1999).
- **Seminal paper identifying tetrahydrobiopterin (BH4)-responsiveness in patients with PKU.**
- 33 Fiege B, Blau N. Assessment of tetrahydrobiopterin (BH4) responsiveness in phenylketonuria. *J. Pediatr.* 150(6), 627–630 (2007).
- **Large study of BH4 responsiveness in the newborn and children.**
- 34 LaClair CE, Ney DM, MacLeod EL, Etzel MR. Purification and use of glycomacropeptide for nutritional management of phenylketonuria. *J. Food Sci.* 74(4), E199–E206 (2009).
- 35 Matalon R, Surendran S, Matalon KM *et al.* Future role of large neutral amino acids in transport of phenylalanine into the brain. *Pediatrics* 112(6 Pt 2), 1570–1574 (2003).
- 36 Sarkissian CN, Shao Z, Blain F *et al.* A different approach to treatment of phenylketonuria: phenylalanine degradation with recombinant phenylalanine ammonia lyase. *Proc. Natl Acad. Sci. USA* 96(5), 2339–2344 (1999).
- 37 Ding Z, Harding C, Rebuffat A, Elzaouk L, Wolff J, Thony B. Correction of murine PKU following AAV-mediated intramuscular expression of a complete phenylalanine hydroxylating system. *Mol. Ther.* 16(4), 673–681 (2008).
- 38 Harding C. Progress toward cell-directed therapy for phenylketonuria. *Clin. Genet.* 74(2), 97–104 (2008).
- 39 Yildirim S, Tokatli A, Yilmaz E, Coskun T. Assessment of tetrahydrobiopterin responsiveness in Turkish hyperphenylalaninemic patients. *Turk. J. Pediatr.* 49(1), 1–6 (2007).
- 40 Zurfluh MR, Zschocke J, Lindner M *et al.* Molecular genetics of tetrahydrobiopterin-responsive phenylalanine hydroxylase deficiency. *Hum. Mutat.* 29(1), 167–175 (2008).
- **Review of genetic mutations associated with BH4 responsiveness in PKU.**
- 41 Burton BK, Grange DK, Milanowski A *et al.* The response of patients with phenylketonuria and elevated serum phenylalanine to treatment with oral sapropterin dihydrochloride (6R-tetrahydrobiopterin): a Phase II, multicentre, open-label, screening study. *J. Inherit. Metab. Dis.* 30(5), 700–707 (2007).
- **Large-scale screening trial of BH4 responsiveness.**
- 42 Karacić I, Meili D, Sarnavka V *et al.* Genotype-predicted tetrahydrobiopterin (BH4)-responsiveness and molecular genetics in Croatian patients with phenylalanine hydroxylase (PAH) deficiency. *Mol. Genet. Metab.* 97(3), 165–171 (2009).
- 43 Thony B, Blau N. Mutations in the BH4-metabolizing genes GTP cyclohydrolase I, 6-pyruvoyl-tetrahydropterin synthase, sepiapterin reductase, carbinolamine-4a-dehydratase, and dihydropteridine reductase. *Hum. Mutat.* 27(9), 870–878 (2006).
- **Comprehensive summary of genetic mutations associated with BH4 deficiencies.**
- 44 Blau N, Bonafe L, Blaskovics M. Disorders of phenylalanine and tetrahydrobiopterin metabolism. In: *Physician's Guide to the Laboratory Diagnosis of Metabolic Diseases*. Blau N, Duran M, Blaskovics M, Gibson KM (Eds). Springer, Heidelberg, Germany 89–106 (2005).
- 45 Blau N, Erlandsen H. The metabolic and molecular bases of tetrahydrobiopterin-responsive phenylalanine hydroxylase deficiency. *Mol. Genet. Metab.* 82(2), 101–111 (2004).
- 46 Pey AL, Perez B, Desviat LR *et al.* Mechanisms underlying responsiveness to tetrahydrobiopterin in mild phenylketonuria mutations. *Hum. Mutat.* 24(5), 388–399 (2004).
- 47 Gersting SW, Kemter KF, Staudigl M *et al.* Loss of function in phenylketonuria is caused by impaired molecular motions and conformational instability. *Am. J. Hum. Genet.* 83(1), 5–17 (2008).
- 48 Erlandsen H, Pey AL, Gamez A *et al.* Correction of kinetic and stability defects by tetrahydrobiopterin in phenylketonuria patients with certain phenylalanine hydroxylase mutations. *Proc. Natl. Acad. Sci. USA* 101(48), 16903–16908 (2004).

- 49 Hayashi T, Ogata A, Takeshima M *et al.* Studies on the metabolism and disposition of sapropterin hydrochloride (SUN 0588) L-erythro-tetrahydrobiopterin hydrochloride in rats. *Kisho to Rinsho (Basic and Clinical Medicine)* 26(8), 1–31 (1992).
- 50 Feillet F, Clarke L, Meli C *et al.* Pharmacokinetics of sapropterin in patients with phenylketonuria. *Clin. Pharmacokinet.* 47(12), 817–825 (2008).
- 51 Fiege B, Ballhausen D, Kierat L *et al.* Plasma tetrahydrobiopterin and its pharmacokinetic following oral administration. *Mol. Genet. Metab.* 81(1), 45–51 (2004).
- 52 Shintaku H, Kure S, Ohura T *et al.* Long-term treatment and diagnosis of tetrahydrobiopterin-responsive hyperphenylalaninemia with a mutant phenylalanine hydroxylase gene. *Pediatr. Res.* 55(3), 425–430 (2004).
- 53 Levy HL, Milanowski A, Chakrapani A *et al.* Efficacy of sapropterin dihydrochloride (tetrahydrobiopterin, 6R-BH4) for reduction of phenylalanine concentration in patients with phenylketonuria: a Phase III randomised placebo-controlled study. *Lancet* 370(9586), 504–510 (2007).
- 54 Musson DG, Kramer WG, Foehr E *et al.* Relative bioavailability of sapropterin from intact and dissolved sapropterin dihydrochloride tablets and the effects of food: a randomized, open-label, crossover study in healthy adults. *Clin. Ther.* 32(2), 338–346 (2010).
- 55 Kuster T, Matasovic A, Niederwieser A. Application of gas chromatography-mass spectrometry to the study of biopterin metabolism in man. Detection of biolumazine and 2'-deoxysepialumazine. *J. Chromatogr.* 290, 303–310 (1984).
- 56 Niederwieser A, Matasovic A, Kuster T, Staudenmann W, Pfeleiderer W, Scheibenreiter S. Catabolism of tetrahydrobiopterin in man. In: *Chemistry and Biology of Pteridines*. Cooper BA, Whitehead VM (Eds). Walter de Gruyter, Berlin, Germany 305–308 (1986).
- 57 Hoshiga M, Hatakeyama K, Watanabe M, Shimada M, Kagamiyama H. Autoradiographic distribution of [¹⁴C tetrahydrobiopterin and its developmental change in mice. *J. Pharmacol. Exp. Ther.* 267(2), 971–978 (1993).
- 58 Nielsen JB, Nielsen KE, Guttler F. Tetrahydrobiopterin responsiveness after extended loading test of 12 Danish PKU patients with the Y414C mutation. *J. Inherit. Metab. Dis.* 33(1), 9–16 (2010).
- 59 Wasserstein M, Burton B, Cederbaum S *et al.* Results of a Phase II, multicenter, open-label study of sapropterin dihydrochloride in subjects with hyperphenylalaninemia related to primary BH4 deficiency. Presented at: *The Annual Meeting of the American Society for Human Genetics (ASHG)*, 2008. PA, USA, 11–15 November 2008.
- 60 Lee P, Treacy EP, Crombez E *et al.* Safety and efficacy of 22 weeks of treatment with sapropterin dihydrochloride in patients with phenylketonuria. *Am. J. Med. Genet. A* 146A(22), 2851–2859 (2008).
- **Long-term Phase III study of sapropterin.**
- 61 Trefz FK, Burton BK, Longo N *et al.* Efficacy of sapropterin dihydrochloride in increasing phenylalanine tolerance in children with phenylketonuria: a Phase III, randomized, double-blind, placebo-controlled study. *J. Pediatr.* 154(5), 700–707 (2009).
- **Phase III trial of sapropterin in children.**
- 62 Fernhoff PM, Burton B, Nowacka M, Hennerman J, Kakkis ED, Dorenbaum A. PKU 008: a long-term, open-label study of sapropterin dihydrochloride (Kuvan®) in PKU subjects. Presented at: *The American College of Medical Genetics Annual Meeting*. FL, USA, 25–29 March 2009.
- 63 Champigneulle A, Gasteyer C, Bettiol E, Villemagne H, Nespithal K, Bassett R. The Kuvan® Adult Maternal Pediatric European Registry (KAMPER): a long-term observational study of patients with hyperphenylalaninemia treated with Kuvan®. Presented at: *The 11th International Congress on Inborn Errors of Metabolism*. CA, USA, 29 August–2 September 2009 (Abstract 195).
- 64 Kurczynski T, Nicely H, Wuebbels B, Reilly D, Pallansch P. Long-Term observations of PKU patients treated with Kuvan®: an introduction to the PKUDOS registry; and its subregistry PKU MOMS for pregnant or nursing PKU patients [abstract]. Presented at: *The American College of Medical Genetics Annual Meeting*. FL, USA, 25–29 March 2009.
- 65 Asubio. Asubio Pharma Company. Press release (2008).
- 66 Blau N, Belanger-Quintana A, Demirkol M *et al.* Management of phenylketonuria in Europe: Survey results from 19 countries. *Mol. Genet. Metab.* 99(2), 109–115 (2009).
- **Key survey of current treatment practice of PKU across Europe.**
- 67 Blau N. Defining tetrahydrobiopterin (BH4)-responsiveness in PKU. *J. Inherit. Metab. Dis.* 31(1), 2–3 (2008).
- 68 Levy H, Burton B, Cederbaum S, Scriver C. Recommendations for evaluation of responsiveness to tetrahydrobiopterin (BH(4)) in phenylketonuria and its use in treatment. *Mol. Genet. Metab.* 92(4), 287–291 (2007).
- 69 Muntau AC, Röschinger W, Habich M *et al.* Tetrahydrobiopterin as an alternative treatment for mild phenylketonuria. *N. Engl. J. Med.* 347, 2122–2132 (2002).
- 70 Ponzzone A, Porta F, Mussa A, Alluto A, Ferraris S, Spada M. Unresponsiveness to tetrahydrobiopterin of phenylalanine hydroxylase deficiency. *Metabolism* 59, 645–652 (2009).

Websites

- 101 Phenylalanine hydroxylase locus knowledge base
www.pahdb.mcgill.ca
- **Comprehensive up-to-date database of genetic mutations at the PAH locus.**
- 102 Databases of pediatric neurotransmitter disorders, including database of BH4-responsive PAH genotypes
www.biopku.org
- **Comprehensive database of genotypes and loading test data.**
- 103 NSPKU. Management of PKU: a consensus document for the diagnosis and management of children, adolescents and adults with phenylketonuria (2004)
www.nspku.org/Documents/Management%20of%20PKU.pdf
- 104 US FDA. Kuvan Prescribing Information (2007)
www.kuvan.com
(Accessed February 2010)
- 105 EMEA. Kuvan EU Summary of Product Characteristics (2008)
www.emea.europa.eu
(Accessed February 2010)
- 106 Daiichi Sankyo Group Report (2009)
www.daiichisankyo.com/corporate/report/pdf/2009/g_ds_all_2009.pdf
- 107 EMA. CHMP Assessment Report for Kuvan (2008)
www.emea.europa.eu/humandocs/PDFs/EPAR/kuvan/H-9/l-en6.pdf
- 108 BioMarin Pharmaceuticals (2010)
http://phx.corporate-ir.net/phoenix.zhtml?c=106657&p=irol-newsArticle&ID=139/110&highlight