PKU and the Brain: A 2023 Update

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Table 1. List of Abbreviations

Abbreviations	Full names
PKU	Phenylketonuria
BH_4	Tetrahydrobiopterin
ACMG	American College of Medical Genetics and Genomics
Phe	Phenylalanine
Tyr	Tyrosine
PAH	Phenylalanine hydroxylase
MPD	Mild hyperphenylalaninemia
ADHD	Attention deficit hyperactivity disorder
PEGlylated-PAL	Polyethylene-glycolylated phenylalanine ammonia-lyase
HD-Ad vectors	Helper-dependent adenoviral vectors
rAAV	Recombinant adeno-associated viruses
CRISPR/Cas9	Clustered regularly interspaced short palindromic repeats/CRISPR associated protein-9

KUVAN® (sapropterin) is a man-made/synthetic form of the cofactor tetrahydrobiopterin (BH_4). KUVAN® is the first approved drug for phenylketonuria (PKU) by Health Canada.

According to the American College of Medical Genetics and Genomics (ACMG) guideline, people with PKU usually take 5-20 mg/kg of sapropterin by month every day.[1] The starting dose of sapropterin depends on age. For kids between 1 month and 6 years old, the recommended starting dose is 10 mg/kg once daily.[1,2] For teens older than 7 years and adults, the recommended starting dose is from 10 to 20 mg/kg once daily.[1,2] People with PKU can take sapropterin with water, apple juice, or soft food, such as apple sauce and pudding.[2] People with PKU must get a prescription of the specific dosage from a PKU physician before taking sapropterin.[2] In addition, people with PKU must adjust the sapropterin dose according to blood phenylalanine (Phe) levels if their physician tells them to do so.[1,2] The dose can increase to 20 mg/kg daily if blood Phe levels do not decrease compared to before taking sapropterin.[1,2]

Some people on sapropterin, especially children, have vitamin B12 deficiency.[3] This is probably because these children are not strictly following the low-Phe dietary therapy and taking medical foods anymore after taking sapropterin.[3] Therefore, people on sapropterin and their physicians must constantly monitor blood Phe levels for adjusting dietary therapy and medical food consumption.[3]

Clinical trial summary

From 2015 to 2023, eight clinical trials show that sapropterin can lower blood Phe levels in combination with the low-Phe dietary therapy, increase the ability to process more Phe from food, and increase conversion from Phe to tyrosine (Tyr).[4-11] Taking sapropterin at two divided doses (e.g., 10 mg/kg at breakfast and the other 10 mg/kg at dinner) can better increase everyday Phe consumption and ability to process more Phe from food than a single daily dose (e.g., 20 mg/kg all at once).[8]

Furthermore, sapropterin can improve inattentive symptoms and hyperactivity of attention deficit hyperactivity disorder (ADHD).[12,13] ADHD inattentive symptoms and hyperactivity are tested by the ADHD Self-Reported Scale inattentive subscale.[12] Lower scores mean less inattentive symptoms and hyperactivity.[12] ADHD Self-Reported Scale inattentive subscale scores start reducing from 4 weeks of taking sapropterin.[12] Furthermore, executive functioning improves in sapropterin-treated children and adolescents from 8 to 17 years old.[12,13] The improved executive functioning includes inhibition, shift, emotional control, initiation, working memory, planning, material organizing, and monitoring (Table 2).[12,13]

For people from 1 month to 50 years old, the common adverse reactions to sapropterin (incidence ≥4%) are headache, diarrhea, vomiting, cough, nasal obstruction, runny nose, and throat pain.[2] Sapropterin is safe to use based on up to 15 years of follow-up study in the United States. This 15-year study shows that sapropterin-related adverse events occur in 6% of sapropterin-treated individuals across all ages.[11]

Most (91%) of these adverse events are not serious.[11] The most common types are digestive disorders (3%), nervous system disorders (2%), and respiratory, thoracic and mediastinal disorders (0.3%).[11]

However, only 25 to 50% of individuals with PKU are responsive to sapropterin.[1] Scientists tried to predict the responsiveness of each individual with PKU by different genotypes of phenylalanine hydroxylase (PAH) because mutations of PAH are the main cause of PKU.[1] Nevertheless, genotypes of PAH cannot fully predict the responsiveness to sapropterin.[1] Thus, a testing trial usually is conducted to determine the responsiveness of each individual before a physician prescribes sapropterin to them.[1] During the testing trial, participants need to take 20 mg/kg of sapropterin daily for 4 weeks.[1] At the same time, clinicians will measure a baseline blood Phe level right before the first dose of sapropterin is taken.[1] They will also measure blood Phe levels at 24 hours, 1 week, 2 weeks, and 4 weeks during the daily sapropterin treatment.[1] Assuming participants are taking the low-Phe dietary therapy during the testing trial, sapropterin responsiveness is defined as a more than 30% decline in blood Phe levels.[1]

Sepiapterin or PTC293, previously called CNSA-001, can be converted to cofactor BH_4 by two enzymes in our body, sepiapterin reductase and dihydrofolate reductase.

Clinical trial summary

In a small clinical trial with healthy adults, sepiapterin can increase BH_4 levels in the blood and central nervous system.[14,15] Under a low-Phe dietary therapy, blood Phe levels after adults with PKU taking 60 mg/kg of sepiapterin reduce more than those taking 20 mg/kg.[15] Only 60 mg/kg of sepiapterin can decrease blood Phe levels in classical PKU.[15]

Classical PKU is the most severe form of PKU.[16] Classical PKU is caused by complete or near-complete PAH loss and is defined as blood Phe levels higher than 1,200 µmol/L without any treatment.[16] The other types of PKU include atypical/mild PKU and non-PKU mild hyperphenylalaninemia (MPH).[16] Atypical/mild PKU is defined as 600-1200 µmol/L of blood Phe levels without treatment.[16] MPH is defined as 200-600 µmol/L of blood Phe levels without treatment.[16]

A large clinical trial was completed on May 3rd, 2023, and showed 63% mean blood Phe level reduction in 98 sepiapterin-treated adults with PKU compared to those without taking sepiapterin.[17,18] In sepiapterin-treated adults with classical PKU, mean blood Phe reduction is 69% compared to those who did not take sepiapterin.[17,18]

All adverse effects reported are mild or moderate and temporary.[15-18] A large clinical trial testing the safety of taking sepiapterin for the long term has not been completed.[19]

Table 2. Definitions of executive functioning improved by sapropterin.

Executive functioning	Definitions

Inhibition	Ability to resist interference irrelevant to current task or mind state
Shift	Ability to change what does not work and adapt to new situations
Initiation	Ability to get started in doing things
Working memory	Ability to temporarily remember a piece of information
Material organizing	Ability to organize complex learning materials for understanding
Monitoring	Ability to evaluate progress or current mistakes

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Palynziq®/Pegvaliase:

In 2018, PALYNZIQ®, hereinafter referred to as Pegvaliase, was approved by multiple health regulators, including Health Canada as the first enzyme substitution therapy for PKU.[1] Prior to this, Sapropterin was the only enzyme therapy available for the treatment of PKU.[2] Substitution therapies work by replacing other enzymes to decrease blood phenylalanine levels.[3] For example, Pegvaliase is a polyethylene-glycol (PEG)ylated phenylalanine ammonia-lyase (PAL) which converts phenylalanine to ammonia and transcinnamic acid acting as a substitute for PAH activity.[4] Pegvaliase is administered as an injection with customized dosing for the treatment of PKU in patients aged 16 and older who have blood Phe levels above 600 umol/L (10mg/dL) despite dietary management.[4]

Pegvaliase has demonstrated robust efficacy in the reduction of blood Phe levels and improved cognitive symptoms in patients who fail to respond to existing therapies and dietary management.[4] Treatment with Pegvaliase should be directed by physicians experienced in the management of PKU. Each carton contains pre-filled syringes of either 2.5mg, 10mg, or 20mg doses.[4] Dosing can be expected to consist of three phases: induction, titration, and maintenance. In the induction phase, the recommended starting dose is 2.5mg administered once weekly for 4 weeks. In the titration phase, doses are escalated gradually based on patient tolerability to achieve target blood Phe levels as determined by the clinician. In the maintenance phase, the dose is individualized to the patient and can range from 20-60mg daily.[4] Prior to determination of the maintenance dose, dietary Phe intake should remain consistent and blood Phe levels should be monitored once a month with frequency reassessed after establishing a maintenance dose.[4] Generally, it is recommended that Pegvaliase should be discontinued if patients do not respond after 48 weeks of continuous treatment with the maximum dosage of 60mg daily, however, all dosing regimens must be determined by the PKU clinician.[4]

Pegvaliase has been associated with acute systemic hypersensitivity reactions (anaphylaxis), that must be taken into consideration by PKU-specialized healthcare professionals prior to dosing.[5–11] Prior to initial dosing, patients should be trained on recognizing signs and symptoms of anaphylaxis as well as when and how to administer an epinephrine auto-injector or pre-filled syringe/pen if necessary.[4] Initial administration must be performed under the supervision of a healthcare professional and patients should be monitored for at least 1 hour following injection.[4] Following initial supervision, patients may transition to self-administration or caregiver-administration.[4] In the case of self-injection, patients should be monitored by a trained observer for each injection for at least 6 months. Due to the risk for hypersensitivity reactions, premedication with H1- or H2-receptor antagonists and/or antipyretics may be recommended.[4] Patients who have experienced severe systemic hypersensitivity reactions to Pegvaliase (severe serum sickness, angioedema) or recurrent mild to moderate acute systemic reactions (anaphylaxis) should seek medical care and permanently discontinue use.[4] Pegvaliase

is contraindicated in patients who have also previously experienced severe hypersensitivity reactions to other PEGylated products.[4] In the case of re-administration of Pegvaliase, the risks and benefits must first be considered by healthcare professionals.

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SYNB1934 Synlogic

SYNB1934, a designer strain of bacteria and an orally administered therapeutic, presents a new approach to treating PKU with the potential to be the first drug approved as both monotherapy and adjunct therapy.[1] SYNB1934 (non-proprietary name labafenogene marselecobac) is an improved, more potent version of an already existing strain, developed by Synlogic- SYNB1618.[2]

Both are strains of *E.Coli* bacteria, engineered specifically to degrade Phe in the GI tract to reduce the amino acid absorption and subsequent release into the bloodstream. However, the new strain is believed to have a higher capacity for Phe degradation due to its increased levels of PAL enzyme activity.[3] This would mean that, in the new formulation, less drug is required to achieve the same effect, lowering the risk of adverse reactions.

Phase 1 study, concluded in 2021, included 100 healthy volunteers and showed the drug to be safe, allowing it to move on to Phase 2.[4] Phase 2 was a smaller study, run with 20 participants, but focused on recruiting people with PKU to assess efficacy by comparing the older strain, SYNB1618, and the newer one, SYNB1934.[5] Following the promising results, SYNB1934 received fast-track designation, rare disease designation, and orphan drug designation by the FDA.[6] These allow to expedite the review and approval process, once the results from Phase 3 become available.

As of November of 2023, Synlogic, the company behind SYNB1934, is currently recruiting patients for their stage 3 human study of SYNB1934, called Synpheny-3, which was announced in June of 2023. It is a randomized, placebo-controlled, multi-center global study. The study is expected to conclude in October of 2024, with Canadian clinics in Calgary and Hamilton participating in the process. The study includes the option of an open-label extension, where, assuming positive changes, patients will be allowed to remain on the drug for up to three years.[7]

The scientists are looking to recruit about 150 people globally. The goal is to examine both efficacy and safety by running dose-escalation studies to determine the optimal dosage, and randomized withdrawal studies, with half of patients staying on the drug and the other half being transferred to placebo, to track efficacy.[7]
Once the results from Synpheny-3 are analyzed, they are expected to be submitted to Health Regulators for review to determine if SYNB1934 should indeed be approved as a new PKU therapy.

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Gene therapy

The first in vivo study using mice demonstrated that gene therapy using helper-dependent adenoviral (HD-Ad) vectors successfully treated PKU in mice.[1] The therapy resulted in complete normalization of Phe and Tyr levels, reversed coat hypopigmentation for six months, and restored spatial learning deficits, hippocampus receptor levels, and long-term potentiation, which are impaired in PKU mice.[1] This shows that HD-Ad vector-mediated gene therapy holds promise as an effective approach for PKU treatment.[1]

In another approach, in the phase of in vivo trials as well, researchers used an adeno-associated viral vector (AAV8-PAL) to deliver the PAL gene, correcting hyperphenylalaninemia in PKU mice.[2] The study demonstrated long-term correction without liver injury, indicating the potential of AAV-mediated liver delivery of the PAL gene for PKU treatment.[2]

In the last potential therapy going through in vivo studies, a combination of drugs was used and treated hepatocytes of mice with PKU through CRISPR/Cas9 gene editing.[3] This approach restored liver enzyme activity, reduced blood phenylalanine, and prevented maternal PKU effects during breeding, showing the promise of CRISPR/Cas9 for permanent PKU gene editing.[3]

Through many stages of study, including clinical studies, therapy using liver-directed recombinant adeno-associated viruses (rAAV) has become the most advanced and studied gene addition therapy.[4] There are several types of AAV, and the choice of the AAV type could affect the infectivity and the tissue distribution of the vector when injected.[4] While clinical studies in adult PKU patients have shown positive results, reduced levels of vectors in the patient's liver are seen due to hepatocyte turnover.[4] Potential relapse of PKU symptoms can be prevented and dealt with by regular injection of vectors.[4] In addition, since such treatment is rapidly reversed in juvenile animal models, effectiveness in human adolescents is yet to be determined.[4] Lastly, this therapy has the risk of the patient developing immunity against AAV, disabling the patient to receive a follow-up injection to maintain the effect.[4] Although one type of AAV designed by BioMarin Pharmaceutical Corp, AAV5, has been approved by the European Medicines Agency, there are areas of research remaining.[4]

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