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Committee for Medicinal Products for Human Use (CHMP)

CHMP assessment report

Sephience

International non-proprietary name: Sepiapterin

Procedure No. EMEA/H/C/006331/0000

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List of abbreviations

(Q)SAR	Quantitative structure activity relationship
ABC	ATP-binding cassette
ADD	Additive residual error
ADME	Absorption, distribution, metabolism, and excretion
ADR	Adverse drug reaction
AE	Adverse event
ALP	Alkaline phosphatase
AME	Absorption, metabolism, and excretion
AP	Applicant's Part (or Open Part) of a DMF
API	Active Pharmaceutical Ingredient
AR	(1) Aldose reductase; (2) Assessment Report
ASM	Active Substance Manufacturer
ATC	Anatomical Therapeutic Chemical (code)
AUC	Area under the concentration-time curve
AUC_{0-24h}	Area under the plasma concentration curve from 0 to 24 hours postdose
AUC_{0-48h}	Area under the plasma concentration curve from 0 to 48 hours postdose
AUC_{0-inf}	Area under the concentration-time curve from 0 to infinity (extrapolated)
AUC_{0-t}	Area under the plasma concentration curve from 0 to the last measured plasma concentration
BBB	Blood-brain barrier
BCRP	Breast cancer resistance protein
BDC	Bile duct cannulated
BE	Bioequivalence
BH_2	Dihydrobiopterin or 7,8-dihydrobiopterin
BH_4	Tetrahydrobiopterin
BH_4-OH	4α-hydroxy-tetrahydrobiopterin
BMI	Body mass index
BQL	Below the quantifiable limit
BSEP	Bile salt export pump
BSV	Between subject variability
CAS	Chemical abstract service

CEP	Certificate of Suitability of the EP
CFU	Colony Forming Units
CHMP	Committee for Medicinal Products for Human Use
CI	Confidence interval
CIOMS	Council for International Organizations for Medical Sciences
CL/F	Apparent systemic clearance
CNS	Central nervous system
CoA	Certificate of Analysis
CoS	Certificate of Suitability
cPKU	Classical phenylketonuria/PKU
CPP	Critical process parameter
CQA	Critical quality attribute
C-QTc	Concentration-QT corrected interval
CR	Carbonyl reductase
CRS	Chemical Reference Substance (official standard)
CSF	Cerebrospinal fluid
CSR	Clinical study report
CTCAE	Common Terminology Criteria for Adverse Events
CV	Cardiovascular
CYP	Cytochrome P450
CYP450	Cytochrome P450
DAD	Diode array detection
DALA	Drug abuse liability assessment
DBS	Dried blood sample
DCoH1	Dimerization cofactor of hepatocyte nuclear factor 1α
DDI	Drug-drug interaction
DHFR	Dihydrofolate reductase
DHPR	Dihydropteridine reductase
DMF	Drug Master File = Active Substance Master File
DoE	Design of experiments
DP	Decentralised (Application) Procedure

DP	Drug product
DRF	Dose range-finding
DS	Drug substance
DSC	Differential Scanning Calorimetry
DTT	Dithiotreitol
DVS	Dynamic vapor sorption
EAIR	Exposure-adjusted incidence rate
ECG	Electrocardiogram
EDQM	European Directorate for the Quality of Medicines
EEA	European Economic Area
EFD	Embryo-foetal developmental
eGFR	Estimated glomerular filtration rate
EMA	European Medicines Agency
ENT	Equilibrative nucleoside transporter
EOS	End of study
EPAR	European Public Assessment Report
EQ-5D	European Quality of Life – 5 Dimensions
ETV	Early Termination Visit
EU	European Union
EuPFI	European Paediatric Formulation Initiative
F	Female
FAS	Full Analysis Set
FEED	Fertility and early embryonic development
FOB	Functional observation battery
FPFV	First patient first visit
FT-IR	Fourier transform-infrared spectroscopy
FUQ	follow-up questionnaire
GD	Gestation day
GERD	Gastroesophageal reflux disease
GLP	Good Laboratory Practice
GI	Gastrointestinal

GTP	Guanosine triphosphate
GTPCH	Guanosine triphosphate cyclohydrolase;
H_2N	Azanide
HDPE	High Density Polyethylene
hERG	Human ether-à-go-go related gene
HIAA	5-hydroxyindoleactic acid
HMG-CoA	3-hydroxy-3-methylglutaryl-coenzyme A
HPA	Hyperphenylalaninemia
HPLC	High performance liquid chromatography
HSDH2	3α-hydroxysteroid dehydrogenase type 2
HVA	Homovanillic acid
IC50	Half maximal inhibitory concentration
IKr	Rapid delayed rectifier potassium current
Imax	Maximum inhibition
INN	International Non-proprietary Names
IPC	In-process control
IQ	Intelligence quotient
IR	Infrared
ITT	Intent-to-treat
IU	International Units
JPN	Japanese
LAT1	L-amino acid transporter 1
LDPE	Low Density Polyethylene
LLOQ	Lower limit of quantitation
LNAA	Large neutral amino acid
LOA	Letter of Access
LOD	Limit of Detection
LOQ	(1) Limit of Quantification, (2) List of Questions
LPLV	Last patient last visit
LS	Least squares
LSM	Least squares mean

M	Male
MA	Marketing Authorisation
MAD	Mutual Acceptance of Data
MAH	Marketing Authorisation holder
MATE	Multidrug and toxin extrusion
Max	Maximum
MDR1	Multidrug resistance protein 1
Min	Minimum
MMRM	Mixed model for repeated measures
MS	Mass Spectrometry
MTD	Maximum tolerated dose
N	Number of subjects
n	Number of subjects with events
n.e.	Non-enzymatic
NA	Not applicable
NADPH	Nicotinamide adenine dinucleotide phosphate
NC	Not conducted
NCS	Not clinically significant
ND	Not detected
NLT	Not less than
NMR	Nuclear magnetic resonance
NMT	Not more than
NO	Nitric oxide
NOAEL	No observed adverse effect level
NOEL	No observed effect level
NOS	Nitric oxide synthase
Not PT	Not Phe tolerance
NR	Normal range
NZW	New Zealand White
OAT	Organic anion transporter
OATP	Organic anion transporting polypeptide

OCT	Organic cation transporter
OECD	Organisation for Economic Co-operation and Development
OFV	Objective function value
OOS	Out of Specifications
P	Part
PAH	Phenylalanine hydroxylase
PAL	Phenylalanine ammonia lyase
PBD	Primary tetrahydrobiopterin/BH_4 deficiency
PBT	Persistent, Bioaccumulative and Toxic
pbo	Placebo
PCD	Pterin-4α-carbinolamine dehydratase
PD	Pharmacodynamic(s)
P-gp	P-glycoprotein
PDE	Permitted Daily Exposure
PE	Polyethylene
PET	Polyethylene terephthalate
Ph.Eur.	European Pharmacopoeia
Phe	Phenylalanine
PIL	Patient Information Leaflet
PIP	Paediatric Investigational Plan
PK	Pharmacokinetic(s)
PKU	Phenylketonuria
PKU-QOL	Phenylketonuria quality of life
PL	Package leaflet
PND	Postnatal day
PP	(1) Process parameter; (2) Polypropylene
PPND	Pre- and postnatal development
PSUR	Periodic safety update report
PT	Preferred term
PT	Phe tolerance
PTPS	6-pyruvoyl-tetrahydropterin synthase

PVC	Poly vinyl chloride
PY	Patient-years
QOL	Quality of life
QOS	Quality Overall Summary
QTcF	Fridericia's corrected QT
rAvPAL	Recombinant phenylalanine ammonia lyase
RDA	Recommended daily allowance
RH	Relative humidity
RMP	Risk Management Plan
RP	Restricted Part (or Closed Part) of a DMF
RPC	randomised placebo-controlled
rpm	Revolutions per minute
RRT	Relative retention time
RSD	Relative standard deviation
SAD	Single ascending dose
SAE	Serious adverse event
SAP	Statistical analysis plan
SAWP	Scientific Advice Working Party
SCS	Summary of Clinical Safety
SD	Standard deviation
SE	Standard error
SmPC	Summary of Product Characteristics
SOC	System organ class
SP	Sepiapterin
SPC	Summary of Product Characteristics
SR	Sepiapterin reductase
TEAE	Treatment-emergent adverse event
TGA	Thermo-Gravimetric Analysis
TH	Tyrosine hydroxylase
TK	Toxicokinetic(s)
T_{max}	Time to maximal observed plasma concentration

TPH	Tryptophan hydroxylases
TRA	Total radioactivity
Trp	Tryptophan
TTC	Threshold of toxicological concern
Tyr	Tyrosine
UK	United Kingdom
US	United States
UV	Ultraviolet
UV-Vis	Ultraviolet visible spectroscopy
Vc/F	Apparent central volume of distribution
Vp/F	Apparent peripheral volume of distribution
W	Week
Wk	Week
WOE	Weight of evidence
XRD	Single-crystal X-ray diffraction
XRPD	X-ray powder diffraction
ΔQTcF	QTcF Change from baseline

* General list of abbreviations. Not all of them will be used.

1. Background information on the procedure

1.1. Submission of the dossier

The Applicant PTC Therapeutics International Limited submitted on 28 March 2024 an application for marketing authorisation to the European Medicines Agency (EMA) for Sephience, through the centralised procedure falling within the Article 3(1) and point 4 of Annex of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 26 April 2023.

Sephience was designated as an orphan medicinal product EU/3/21/2435 on 20 May 2021 in the following condition: treatment of hyperphenylalaninaemia.

The Applicant applied for the following indication: Sephience is indicated for the treatment of hyperphenylalaninaemia (HPA) in adult and paediatric patients with phenylketonuria (PKU).

1.2. Legal basis, dossier content

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC - complete and independent application

The application submitted is composed of administrative information, complete quality data, non-clinical and clinical data based on Applicants' own tests and studies and/or bibliographic literature substituting/supporting certain test(s) or study(ies).

1.3. Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision(s) P/0041/2024 on the agreement of a paediatric investigation plan (PIP).

At the time of submission of the application, the PIP P/0041/2024 was not yet completed as some measures were deferred.

1.4. Information relating to orphan market exclusivity

1.4.1. Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the Applicant did submit a critical report addressing the possible similarity with authorised orphan medicinal products.

1.5. Applicant's request(s) for consideration

1.5.1. Accelerated assessment

The Applicant requested accelerated assessment in accordance to Article 14 (9) of Regulation (EC) No

726/2004.

1.5.2. New active Substance status

The Applicant requested the active substance sepiapterin contained in the above medicinal product to be considered as a new active substance in comparison to sapropterin previously authorised in the European Union as Kuvan, as the Applicant claimed that sepiapterin differs significantly in properties with regard to safety and/or efficacy from the already authorised active substance.

1.6. Protocol assistance

The applicant received the following Protocol assistance on the development relevant for the indication subject to the present application:

Date	**Reference**	**SAWP co-ordinators**
29 January 2021	EMEA/H/SA/4715/1/2020/III	*Armin Koch, Markku Pasanen, Kolbeinn Gudmundsson and Brigitte Schwarzer-Daum*
24 June 2021	EMA/SA/0000059093	*Johannes Hendrikus Ovelgönne and Brigitte Schwarzer-Daum*
16 September 2021	EMA/SA/0000063706	*Brigitte Schwarzer-Daum and Ferran Torres*
23 February 2023	EMA/SA/0000123467	*Dina Apele-Freimane and Audrey Sultana*

The Protocol assistance pertained to the following quality, non-clinical, and clinical aspects:

Quality:

- Data to support claims for new active substance.
- Acceptability of the proposed starting materials.

Non-clinical:

- Juvenile PK study and waiver of carcinogenicity and reproductive toxicity studies.
- Adequacy of the neonatal non-clinical PK bridging study to support the administration of PTC923 to paediatric patients <12 months of age.

Clinical:

- Primary endpoint in the Phase 3 PTC923-MD-003-PKU study and the bioanalytical method to support its measurement.
- Comparator, treatment period and inclusion criteria including children and newborn infants of all ages in the Phase 3 study.
- Sample size and the statistical approach to assessing efficacy and handling intercurrent events.
- Waiver of a thorough QT study.
- Acceptability of the safety database and the overall clinical development plan for MAA.
- Acceptability of using dried blood sampling via VAMS technology to measure blood Phe for the planned pivotal Phase 3 study.

1.7. Steps taken for the assessment of the product

The Rapporteur and Co-Rapporteur appointed by the CHMP were:

Rapporteur: Fátima Ventura Co-Rapporteur: Alexandre Moreau

The application was received by the EMA on	28 March 2024
The procedure started on	23 May 2024
The CHMP Rapporteur's first Assessment Report was circulated to all CHMP and PRAC members on	12 August 2024
The CHMP Co-Rapporteur's first Assessment Report was circulated to all CHMP and PRAC members on	27 August 2024
The PRAC Rapporteur's first Assessment Report was circulated to all PRAC and CHMP members on	26 August 2024
The CHMP agreed on the consolidated List of Questions to be sent to the Applicant during the meeting on	19 September 2024
The Applicant submitted the responses to the CHMP consolidated List of Questions on	20 December 2024
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Questions to all CHMP and PRAC members on	6 January 2025
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	13 February 2025
The CHMP agreed on a list of outstanding issues to be sent to the Applicant on	27 February 2025
The Applicant submitted the responses to the CHMP List of Outstanding Issues on	26 March 2025
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP and PRAC members on	9 April 2025
The CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a marketing authorisation to Sephience on	25 April 2025
The CHMP adopted a report on similarity of Palynziq on (see Appendix on similarity)	25 April 2025
Furthermore, the CHMP adopted a report on New Active Substance (NAS) status of the active substance contained in the medicinal product (see Appendix on NAS)	25 April 2025

2. Scientific discussion

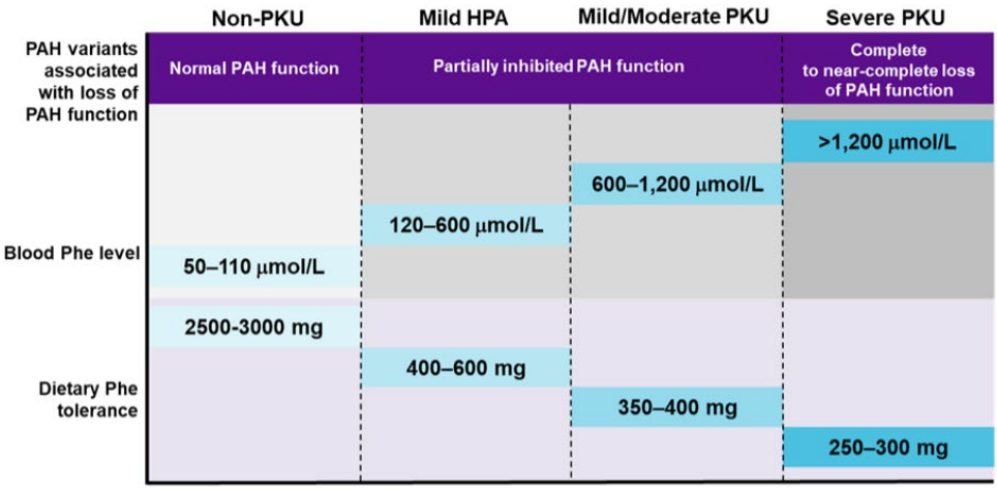
2.1. Problem statement

2.1.1. Disease or condition

The proposed indication is the treatment of hyperphenylalaninaemia (HPA) in adult and paediatric patients with Phenylketonuria (PKU). PKU is a rare, serious, autosomal-recessive inborn error of phenylalanine (Phe) metabolism, characterized by a deficiency and/or reduced functionality of phenylalanine hydroxylase (PAH), which metabolizes Phe to tyrosine, with tetrahydrobiopterin (BH_4) as an essential cofactor (Scriver and Kaufman 2001, Al Hafid and Christodoulou 2015). A reduction in PAH activity due to the expression of PAH variants in patients with PKU results in intolerance to the dietary intake of the essential amino acid and is associated with an elevated blood Phe concentration that can cause a spectrum of symptoms including neurocognitive deficits (Blau and Longo 2015).

PKU, known as "classical PKU" (cPKU), have highly elevated blood Phe concentrations (≥1200 µmol/L) that, if left untreated, is a significant risk factor for severe irreversible neurological dysfunction and intellectual disability with impaired cognitive function (Scriver and Kaufman 2001, Waisbren 2007). The severity of PKU is defined by blood Phe concentration, dietary Phe tolerance, and PAH function (Figure 1).

Figure 1: **Classification of PKU Disease Severity**



Abbreviations: HPA, hyperphenylalaninaemia; PAH, phenylalanine hydroxylase; Phe, phenylalanine; PKU, phenylketonuria; Note: Daily Phe tolerance values presented are for adults.

Source: Adapted from (Blau 2010, Hillert 2020)

2.1.2. Epidemiology and screening tools

The mean incidence of all hyperphenylalaninaemias (HPAs) detected by newborn screening programs in Europe is estimated to be approximately 1:10000 (van Wegberg 2017). In most cases (98% of patients), HPA results from mutations in the *PAH* gene (PKU and variants), while the remaining HPA cases are largely attributable to BH_4 deficiencies. The prevalence of HPA is calculated to be between 0.09/10000 and 2.55/10000 in Europe and

varies depending on the geographical region and population (Hillert 2020, Elhawary 2022), with PKU representing 98% of all HPAs reported (Ho and Christodoulou 2014).

A comparison of genotypes and metabolic phenotypes from >16000 affected patients has revealed differences in disease severity in 51 countries from 17 world regions, with a global distribution of 62% with cPKU, 22% with mild to moderate PKU, and 16% with mild HPA (Hillert 2020).

2.1.3. Biologic features, aetiology and pathogenesis

Phenylalanine hydroxylase (PAH) is a 3-domain protein that is organized as an asymmetric dimer of dimers. This complicated structure permits fine-tuned regulation involving substrate activation, modulation of oligomerization, and modulation of the affinity for the substrate and the cofactor. However, this structural flexibility may render PAH particularly susceptible to protein misfolding, which has deleterious effects on PAH function.

The pathophysiology of PKU is primarily attributed to elevated blood concentrations of Phe and metabolites such as phenylpyruvate. Under normal circumstances, dietary Phe and endogenous protein is metabolized to tyrosine (Tyr) by PAH with tetrahydrobiopterin (BH_4) as a cofactor (Kaufman 1958). However, in PKU where the PAH is not functioning, excess blood Phe is instead converted to highly neurotoxic ketones, including phenylpyruvate, phenylacetate, and phenyllactate (Kaufman 1989). Additionally, elevated Phe concentration in the brain leads to reduced synaptic plasticity/atrophy and impairment of oligodendrocyte myelination (Ashe 2019). Phe competes with the other large neutral amino acids for the same L-type carrier to cross the blood-brain barrier. In addition, with the decrease in circulating Tyr, the synthesis of neurotransmitters such as dopamine, noradrenaline, and adrenaline diminishes (Burlina 2000, Pilotto 2019).

The effects of elevated blood Phe concentration on the central nervous system and resultant cognitive function deficits are proportional to Phe concentration and to the stage of brain growth and development. If left untreated, HPA leads to progressive developmental delays and severe and irreversible intellectual disability. Other neurologic symptoms such as aberrant behaviour, deficits in executive functioning, psychiatric symptoms, and memory impairment may also develop at any time in affected individuals (Blau 2010). In patients with PKU, every 4-week delay in starting treatment results in a decline of approximately 4 IQ points, which underscores that neurological damage as a result of elevated blood Phe concentration starts soon after birth (Smith 1990).

2.1.4. Clinical presentation, diagnosis and prognosis

In patients with PKU on an unrestricted diet or not adequately controlled, sustained elevation of blood Phe concentration above normal levels occur which is neurotoxic. There is a direct association between elevated blood Phe concentration and a range of symptoms including the development of neurocognitive deficits, severe and irreversible intellectual disability, memory impairment, and psychiatric and behavioural problems even in adolescence and adults with the disease (Waisbren 2007, Ashe 2019, Kaufman 1989, Channon 2007, Moyle 2007, VanZutphen 2007, Feldmann 2019).

PKU is diagnosed at birth as part of routine newborn screening. Newborns with PKU can appear normal at birth, with symptoms (e.g., fair skin, eczema, seizures, tremors, and hyperactivity) appearing after several months.

While the early diagnosis and dietary management of PKU at birth has become more commonplace, children with PKU still exhibit neurocognitive deficits compared with non-PKU siblings and children in the general

population. The performance of children and adolescents with PKU at school is often adversely affected relative to their healthy peers. Children and adolescents with PKU have significantly lower IQ scores compared with control subjects, while a greater percentage of children with PKU have attention deficits, and fine motor and executive function are impaired compared with healthy subjects (Gassio 2008). Despite the early implementation and sustained management of dietary Phe intake, deficits in educational achievement, employment, relationships, and quality of life (QOL) remain (Enns 2010, Ashe 2019, Feldmann 2019).

2.1.5. Management

The mainstay of the management of PKU is the dietary restriction of Phe intake. Nevertheless, long-term adherence to a Phe-restricted diet is challenging, and despite optimal dietary control of Phe intake, clinical outcomes remain suboptimal (Ashe 2019). A Phe-restricted diet is often insufficient to effectively manage blood Phe concentration, and therefore, many patients would benefit from a pharmacological treatment to gain and improve control of blood Phe and to potentially liberalize their diet (van Spronsen 2021).

In Europe, there are currently 2 authorized pharmacological treatments for HPA in patients with PKU: sapropterin (e.g., Kuvan) and pegvaliase (Palynziq), yet, due to significant limitations in both therapies, there remains a significant unmet medical need for PKU patients.

Limitations of sapropterin include a low responder rate (blood Phe reduction >30% in approximately 20% of subjects) with low magnitude of blood Phe reduction (approximately 20%), little to no efficacy in patients with cPKU, and limited data supporting Phe tolerance and ability of patients to liberalize diet.

Limitations of pegvaliase include that it is not indicated as either first-line treatment or for patients <16 years of age. In addition, pegvaliase uses injectable administration and requires a prolonged dose titration phase as well as significant safety and tolerability concerns that limit its utility.

Despite dietary Phe restriction and 2 approved pharmacological treatments, approximately two-thirds of patients with PKU do not achieve a sustained reduction in blood Phe concentration to within the recommended European guidelines (Jurecki 2017).

There is a demonstrated and persistent unmet medical need for safe and more effective pharmacological treatments for PKU. Due to the limitations around currently approved pharmacological treatments, there is a persistent need for a new product that is safe and efficacious in a broad PKU population and can allow for meaningful diet liberalization while controlling blood Phe concentrations within the optimal range.

PKU is diagnosed at birth as part of routine newborn screening and requires the lifetime restriction of dietary Phe commencing within 10 days of birth to mitigate against severe and irreversible neurological damage and dysfunction (Blau 2010, van Spronsen 2017, van Wegberg 2017).

Blood Phe concentration is a European Medicines Agency-accepted surrogate endpoint of efficacy in patients with PKU and should be monitored regularly, with the aim of regulating blood Phe concentration within age-appropriate European target treatment range through the restriction of dietary Phe intake and inclusion of a pharmacological treatment as appropriate, given that most patients do not achieve target Phe levels with diet restriction alone (Table 1).

Table 1: Age-Appropriate European Guidelines for Blood Phe Concentration in Patients With PKU

Patient Group	Target Range for Blood Phe Concentration
≤12 years and women before and during pregnancy	120 to 360 µmol/L
>12 years	120 to 600 µmol/L

Abbreviations: Phe, phenylalanine; PKU, phenylketonuria

Source: (van Wegberg 2017)

Management of PKU through Restriction of Dietary Phe Intake

A direct association has been documented between blood Phe levels obtained on an unrestricted diet and the development of severe intellectual disability (Waisbren 2007). Consequently, the mainstay of management for PKU consists of natural protein restriction and the consumption of specifically designed Phe-free amino acid supplements, which should be initiated within 10 days of birth (Waisbren 2007, Blau 2010, van Spronsen 2017, van Wegberg 2017).

Importantly, the majority of patients with PKU do not achieve a sustained reduction in blood Phe concentration within the recommended range using diet alone (MacDonald 2012). The main challenges of dietary management include (Ho and Christodoulou 2014): Adherence to a Phe-restricted diet, Nutritional deficiencies, Physical and psychosocial impact.

Table 2 Barriers to Long-Term Compliance with the Dietary Management of PKU

Types of Dietary Management	Barriers to Long-Term Dietary Compliance
• Low-protein diet • Phe-free medicinal food and formula • Modified low-protein products • Glycomacropeptide • High concentration of LNAA	• Palatability and lack of variety of PKU diet • Prohibitive costs of medicinal foods • Risk of malnutrition • Increased gastrointestinal issues from disruption of the gut microbiome • Social stigma and socioeconomic factors (eg, anxiety, depression, fatigue)

Abbreviations: LNAA, large neutral amino acid; Phe, phenylalanine; PKU, phenylketonuria

Source: (Macleod and Ney 2010, McWhorter 2022)

Studies consistently show adherence to dietary management of Phe intake decreases with age and is particularly poor in adolescents and adults due to the high personal and socioeconomic burden to affected individuals and their families (Awiszus and Unger 1990, Levy and Waisbren 1994, Wappner 1999, MacDonald 2012). Furthermore, a restrictive diet is often insufficient to manage the disease effectively, and patients commonly still have elevated Phe levels (van Spronsen 2021). In a survey of US PKU clinics, patient adherence to target blood Phe concentrations between 120 and 360 µM was shown to be age dependent, decreasing from 88% for ages 0 to 4 years, 74% for ages 5 to 12 years, 50% for ages 13 to 17 years, 42% for ages 18 to 29 years, and 33% in adults 30 years and older. Consistent results were observed for clinics using 600 µM as the upper Phe target (Jurecki 2017). Similar findings were reported in another study of 330 patients with PKU (Walter 2002).

Pharmacotherapies for Treatment of PKU

The majority of patients with PKU do not achieve a sustained reduction in blood Phe concentration within the recommended range via the restriction of dietary Phe intake alone and inclusion of a pharmacotherapy is

necessary (MacDonald 2012). To date, 2 products have been approved in Europe for the treatment of HPA in patients with PKU based on a reduction in blood Phe concentration, sapropterin (e.g., Kuvan) and pegvaliase (Palynziq), yet there remains a significant unmet medical need for PKU patients.

Sapropterin

Sapropterin is indicated for the treatment of HPA in adults and paediatric patients of all ages with PKU who have been shown to be responsive to such treatment. Sapropterin is an oral formulation of synthetic BH_4, an essential cofactor of PAH (Sawabe 2004, Sawabe 2005, Ohashi 2011). Oral administration of sapropterin results in an increase in intracellular BH_4 concentration, which subsequently mediates an increase in activity of residual PAH and a subsequent reduction in blood Phe concentration. Sapropterin can also act as a pharmacological chaperone to promote the correct folding of the PAH monomer, helping restore and stabilize PAH function against misfolding (van Spronsen 2021). However, since sapropterin is lipophobic, intracellular penetration is restricted (Hasegawa 2005, Smith 2019a). Furthermore, sapropterin is rapidly oxidized to dihydrobiopterin (BH_2) in the plasma and gut and is renally cleared (Sawabe 2008). From data collected in clinical studies, approximately 20% of patients are responsive to sapropterin (defined as $\geqslant$30% reduction in blood Phe), with an average reduction of mean blood Phe of 20% (Burnett 2007, Levy 2007b). Additionally, sapropterin has limited efficacy in patients with cPKU. There are also limited data supporting the ability of sapropterin to increase dietary Phe tolerance and therefore liberalize their restrictive diet.

Pegvaliase

Pegvaliase is indicated for the second-line treatment of PKU in patients $\geqslant$16 years of age who have inadequate control of blood Phe concentration (blood Phe concentration >600 µmol/L) despite prior management with available treatment options (BioMarin 2020).

The use of pegvaliase is limited by significant safety and tolerability concerns. Potential serious side effects with pegvaliase include injection site reactions, severe arthralgia, nausea, hair loss, and anaphylactic reactions. According to the pegvaliase Summary of Product Characteristics (SmPC), hypersensitivity reactions (75%), injection site reactions (93%), and arthralgia (86%) occurred in the majority of subjects treated with pegvaliase (BioMarin 2020). Due to these potentially serious adverse reactions, patients must carry an adrenaline injection device with them, not administer pegvaliase alone for at least the first 6 months of treatment, and must premedicate with an H1-receptor antagonist, H2 receptor antagonist, and antipyretic prior to each dose during induction and titration.

The pegvaliase administration and dosing regimen can be burdensome to patients. Pegvaliase is administered daily via subcutaneous injection and is associated with a challenging and lengthy dose titration regimen over many months (Lah 2022).

Unmet Medical Need

Each of the management and therapeutic options has limitations, and as such, there remains a significant unmet medical need for new medications that are safe and efficacious for HPA in patients with PKU. Limitations include the following:

- **Sapropterin**: a low responder rate (blood Phe reduction >30% in approximately 20% of subjects), low magnitude of blood Phe reduction (approximately 20%), minimal efficacy in patients with cPKU, and limited data supporting Phe tolerance and ability for patients to liberalize diet and still maintain target Phe levels;
- **Pegvaliase**: associated with serious side effects and is indicated only in patients aged 16 years and older;
- **Phe-restricted diet:** poor adherence to Phe-restricted diets and suboptimal management of blood Phe

levels through dietary management have been observed.

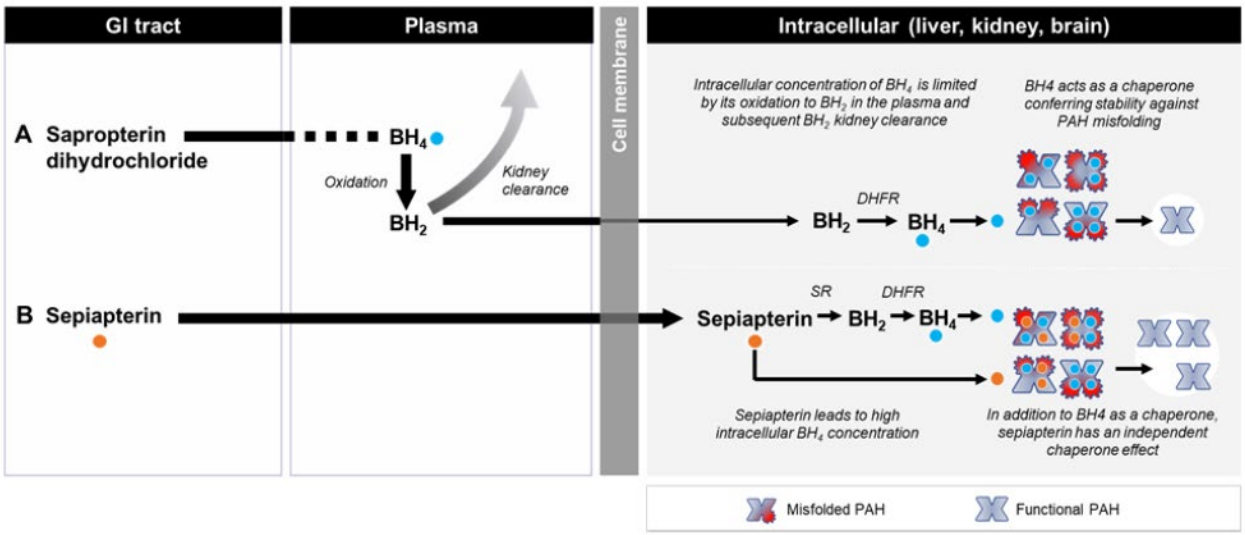
2.2. About the product

To address this persistent unmet medical need for a safe and more efficacious therapy, PTC has developed sepiapterin (7.5, 15, 30, and 60 mg/kg/day based on age), for the treatment of hyperphenylalaninaemia (HPA) in adult and paediatric patients with phenylketonuria (PKU). Sepiapterin is an oral synthetic small molecule equivalent version of biologically produced endogenous sepiapterin and provides a rational approach to treatment of PKU. It can lower blood Phe concentration with the goal of subsequently decreasing PKU disease symptomology and allowing liberalization of diet by acting via two additive mechanisms to elicit a reduction in blood Phe, increasing intracellular BH_4 levels and stabilizing PAH against early intracellular degradation, and ultimately increasing PAH activity (Gersting 2023).

Sepiapterin exerts its pharmacological efficacy via two distinct and additive mechanisms of action (Figure 2). In addition to the rapid conversion of sepiapterin to BH_4, sepiapterin itself exhibits an added chaperone effect on misfolded PAH to improve enzymatic activity and stabilize variant PAH against thermal degradation. The combined chaperone activities of sepiapterin and BH_4 (from sepiapterin metabolism) is a unique property of sepiapterin compared with existing PKU therapies and, therefore, provides an opportunity for sepiapterin to improve treatment by increasing responsiveness and effectively treating a broader PKU population (including patients who are nonresponsive to sapropterin). This is supported by the observation that sepiapterin can restore PAH activity in PKU variants that were not previously responsive to BH_4 treatment (Gersting 2010, Gersting 2023).

In addition, and very importantly, in contrast to lipophobic sapropterin, sepiapterin is actively transported intracellularly, elevating intracellular concentrations of the parent drug and its metabolite, BH_4 (Sawabe 2008, Smith 2019b). The greater bioavailability of sepiapterin increases the ability to restore the function of misfolded PAH, thereby augmenting its enzymatic activity and functional capacity to lower blood Phe concentration.

Figure 2: **Mechanism of Action of sapropterin and sepiapterin**



Abbreviations: BH2, dihydrobiopterin; BH4, tetrahydrobiopterin; DHFR, dihydrofolate reductase; PAH, phenylalanine hydroxylase; SR, sepiapterin reductase

Note: PKU and associated HPA result from pathologic variants within the *PAH* gene resulting in misfolding of the enzyme and reduced enzyme activity. Sepiapterin actively transported into the cell resulting in pharmacologic levels of intracellular BH4

and sepiapterin. Sepiapterin and BH4 increase PAH activity by acting as pharmacologic chaperones to restore conformation structure of the enzyme and consequently promoting conversion of Phe to Tyr.

Source: (Sawabe 2008, Gersting 2010, Smith 2019b)

Sepiapterin crosses membranes, including the blood-brain barrier, and is actively transported into cells more efficiently than BH_4 (Ohashi 2011). This ability facilitates high intracellular concentration of BH_4 in key tissues. This is in contrast to sapropterin where uptake has been shown to be hampered by rapid oxidation to BH_2 and poor penetration into cells, resulting in very low levels of incorporation. Oral synthetic sepiapterin supplementation has major advantages over oral synthetic BH_4 supplementation in terms of gut absorption, stability *in vivo*, sensitivity to oxidation, transport through the cell membrane, and ability to cross the blood-brain barrier (Sawabe 2004, Sawabe 2005).

The ability of sepiapterin to cross the blood-brain barrier was demonstrated in a mouse study, in which elevated BH_4 was observed in the brain following oral administration of sepiapterin (data on file). This finding is supported by results from a Phase 1 study (Study PKU-001) in healthy adults where 60 mg/kg sepiapterin markedly increased levels of BH_4 in the central nervous system (CNS) with little overall effect on CNS levels of already normal neurotransmitter metabolites homovanillic acid (HVA) and 5-hydroxyindoleactic acid (5-HIAA) (Smith 2019a). Furthermore, in 1 subject with low baseline levels, sepiapterin was associated with normalization of cerebrospinal fluid (CSF) levels of BH_4, HVA, and 5-HIAA. As a result, sepiapterin dosing results in a higher intracellular concentration of BH_4 in key tissues such as liver and the brain *versus* a comparable dose of sapropterin. This is important because BH_4 acts as a chaperone to PAH variants and is the essential cofactor necessary for PAH function. Decreased PAH activity, in addition to HPA, leads to hypotyrosinaemia with high Phe concentrations further restricting transport of tyrosine across the blood-brain barrier, impairing tyrosine and tryptophan hydroxylase systems, and leading to decreased concentration of dopamine and serotonin (Ashe 2019). By inducing high intracellular BH_4 concentrations, sepiapterin supplementation results in significant improvement or even normalization of Phe metabolism and the production of neurotransmitters like nitric oxide, dopamine, and serotonin (Sawabe 2008).

Given the positive *in vitro* findings, the potential of sepiapterin as a novel pharmacological treatment for PKU was investigated in clinical studies. The first-in-human healthy volunteer Study PKU-001 demonstrated that dose-related increases in plasma sepiapterin and BH_4 were observed and increases in plasma BH_4 with sepiapterin administration were greater than those obtained with sapropterin administered at the same dose (Module 2.7.2, Section 2.2.1.1) (Smith 2019b).

These data supported further investigation of sepiapterin in patients with PKU. A Phase 2 head-to-head study (PKU-002) in patients with PKU compared the efficacy of 2 oral doses of sepiapterin (20 mg/kg/day and 60 mg/kg/day) and the maximum recommended oral dose of sapropterin (20 mg/kg/day) using a crossover study design. Data from this study showed sepiapterin provided significantly greater, dose-related reduction in blood Phe concentration in patients with PKU compared with sapropterin (Section 4.4.3). It was also demonstrated that sepiapterin was effective at reducing blood Phe in subjects with cPKU, a population in which sapropterin has limited/no efficacy (Bratkovic 2022).

These data supported further clinical development of sepiapterin for the management of PKU and the Phase 3, placebo-controlled, double-blind, global, PTC923-MD-003-PKU study provides evidence of clinically meaningful effect in broad PKU population of children and adults with varying severity of disease (Section 4.4.1). The ongoing Phase 3 long-term, open-label Study PTC923-MD-004-PKU was designed to evaluate the effect of sepiapterin on Phe tolerance, confirm durability of reduction of blood Phe, and establish long-term safety of sepiapterin (Section 4.4.2).

Results from PKU clinical studies demonstrate sepiapterin treatment results in clinically meaningful and durable reductions in blood Phe in patients of all ages and severities of PKU. Sepiapterin provides benefit over existing therapies by effectively reducing blood Phe in patients with PKU who are not effectively treated by other pharmacotherapies. A sustained and durable reduction in blood Phe and tolerance of an increased dietary Phe were demonstrated in subjects who completed Study PTC923-MD-003-PKU and entered the long-term extension Study PTC923-MD-004-PKU.

Sepiapterin is well tolerated and has a consistent and favourable safety profile with continuous exposure of up to 20 months in patients with PKU.

2.3. Type of Application and aspects on development

The CHMP did not agree to the Applicant's request for an accelerated assessment as the product was not considered to be of major public health interest. This was based on the uncertainty at the time of the assessment of the accelerated review request whether the clinical package would enable to conclude on a positive benefit risk ratio in the broad indication claimed by the Applicant, particularly in patients aged under 2 years. In the pivotal PTC923-MD-003-PKU (003), Phase 3 study no patients <1 year old were included and only 3 patients between ⩾1-<2 years were recruited and only participated in Part 1 of the study that was open-label and designed to assess initial responsiveness to sepiapterin; of the 3 patients 2 responded and were directly included in Study 004 to receive open-label sepiapterin. Although the unmet medical need was recognized in a subset of PKU patients who are not adequately controlled or are not eligible to currently approved medicinal products and that lack compliance with the Phe restrictive diet, there was uncertainty on the strength of the clinical data to be submitted in supporting the intended broad indication covering first line treatment of all types of phenylketonuria (PKU) in adult and paediatric patients of all ages. Therefore, CHMP did not consider that, at the stage of the assessment of the accelerated review request, the marketing authorization application constituted a major interest from the point of view of public health and in particular from the viewpoint of therapeutic innovation.

2.4. Quality aspects

2.4.1. Introduction

The finished product is presented as an oral powder containing 250 mg or 1000 mg of sepiapterin as active substance.

Other ingredients are: microcrystalline cellulose (E460), isomalt (E953), mannitol (E491), croscarmellose sodium (E468), xanthan gum (E415), colloidal silicon dioxide or silica colloidal anhydrous (E551), sucralose (E955) and magnesium stearate (E470).

The product is available in heat-sealed laminated aluminium foil sachet: polyethylene terephthalate, white extruded polyethylene (polyester/foil bond), aluminium foil (moisture barrier), and heat-sealed ionomeric resin (adhesive) as described in section 6.5 of the SmPC. Each carton contains 30 unit-dose sachets.

2.4.2. Active Substance

2.4.2.1. General information

The chemical name of sepiapterin is (2-amino-6-[(2*S*)-2-hydroxypropanoyl]-7,8-dihydro-3*H*-pteridin-4-one) corresponding to the molecular formula $C_9H_{11}N_5O_3$. It has a molecular weight of 237.22 g/mol and the following structure:

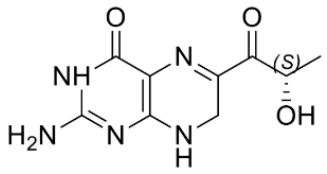


Figure 3: Active substance structure

Sepiapterin is the synthetic analogue of the natural product sepiapterin. The chemical structure of sepiapterin was elucidated by a combination of multinuclear ($^1H/^{13}C$) magnetic resonance spectroscopy, mass spectrometry (MS), Fourier transform-infrared spectroscopy (FT-IR), ultraviolet visible spectroscopy (UV-Vis), chiral high-performance liquid chromatography (HPLC), and polarimetry/optical rotation. The solid state properties of the active substance were measured by X-ray powder diffraction (XRPD), single-crystal X-ray diffraction (XRD), and dynamic vapor sorption (DVS).

The active substance is a yellow to orange powder, moderately hygroscopic. The solubility of sepiapterin was determined in different solvents (<1.3 mg/mL in isopropanol - >48.0 mg/mL in N-Methylpyrolidone) and in water (1.4 mg/mL).

The molecule exhibits polymorphism (Forms A, B, C, D, E, F and G), and it is manufactured and commercialized as crystalline polymorph Form F. Form F is the most stable form known in solid state. Form F has been used in formulation development, in the manufacturing of clinical supplies, and is the intended form for commercial manufacturing.

The following additional general properties have also been described: melting point, optical rotation, pKa, log P, permeability, and hygroscopicity (moderate).

Sepiapterin exhibits stereoisomerism due to the presence of one chiral centre (*S* enantiomer, $[\alpha]^{25}_{589}$ + 92°). Chiral purity is routinely controlled in the active substance specifications.

2.4.2.2. Manufacture, characterisation and process controls

Detailed information on the manufacturing of the active substance has been provided in dossier.

The overall manufacturing process for sepiapterin active substance is described in sufficient detail and involves a six step sequential chemical synthetic procedure, which considers CC-05 as starting material. The starting material is appropriately justified and controlled with acceptable specification.

The overall information provided is sufficient for a thorough assessment. Typical reaction yields for each relevant synthetic step of the active substance were provided.

Six isolated intermediates – two of them isolated as a mixture of diastereomers in the same step – are described in the active substance manufacturing process. Three chemical transformations are involved, one

of them being removal of protecting groups. The last three steps of the active substance manufacturing process are purification steps of the crude active substance.

The proposed starting material CC-05 is synthesized via a custom manufacturing process.

A detailed discussion on potential and actual impurities in the proposed SM CC-05 has been presented, including spiking studies. The analytical methods used to control the proposed starting material CC-05 are described and validated. Validation summaries are presented.

Brought together the control analytical results provided for CC-05 batches manufactured from different precursor suppliers show that the quality profile of CC-05 is equivalent. Likewise, the analytical results for sepiapterin DS manufactured with CC-05 SM obtained from two different precursor suppliers demonstrate that the quality profile of all DS batches tested is comparable, in compliance with the proposed specification.

Specification for all non-compendial reagents, solvents and catalysts listed are given. Specifications of the blanketing nitrogen gas have been provided. A risk assessment was performed to determine the level of residual benzene in the final active substance. Considering that the benzene level does not exceed 30% of the corresponding ICH limit (0.6 ppm) in three consecutive production-scale batches, the Applicant decided to routinely perform skip testing for benzene in sepiapterin active substance. Only one batch will be tested at release annually. All relevant sections have been revised accordingly, and this approach is acceptable.

The non-compendial methods are described and, when relevant, method validation summaries are also included.

The critical steps in the manufacturing process have been identified and all relevant in-process controls are given for all synthetic steps.

Specification and analytical procedures are given for all considered intermediates. The impurity profiles are discussed in detail. Spiking studies have been performed to justify the limits. The analytical method and their validations are described. Batch analyses data are given for all intermediates as well.

As regards process validation and/or evaluation it is stated that process validation for sepiapterin active substance will be completed prior to release of the product to the market. This is acceptable considering that extensive and comprehensive manufacturing process development information provided in the dossier. Operating conditions have been established for the proposed commercial process. The history of both the manufacturing process development as well as of the development history of the analytical methods is presented and discussed.

Equivalent quality of the active substance used in all studies is claimed by the Applicant. Differences in the polymorphic forms used in the preclinical and clinical studies have been appropriately justified. Equivalent quality of the active substance batches from different manufacturers has been demonstrated.

The active substance is packaged in primary sealed LDPE bag/secondary sealed LDPE bag/heat-sealed aluminium foil bag/HDPE drum which complies with Commission Regulation (EU) 10/2011, as amended. The active substance is packaged under nitrogen.

2.4.2.3. Specification

The active substance specification includes tests for: appearance (visual), identity (IR, HPLC-UV), assay (HPLC-UV), individual specified impurities (HPLC-UV), individual unspecified impurities (HPLC-UV), total impurities (HPLC-UV), chiral purity (HPLC-UV), water content (KF), residue on ignition (Ph. Eur.), residual

solvents (GC-HS), crystal form (XRPD), particle size distribution (light diffraction), and microbial limits (Ph. Eur.).

General compendial methods are listed. Both the non-compendial and in-house methods are described: HPLC/UV for the identification, assay and impurities; a dedicated HPLC/UV method for chiral purity; GC/HS for residual solvents. The latter were satisfactorily ICH Q2 validated, with results meeting the acceptance criteria for all evaluated parameters. All methods are adequate to control the substance on a routine basis.

Impurities present at higher than the qualification threshold according to ICH Q3A were qualified by toxicological and clinical studies and appropriate specifications have been set. The discussion is comprehensive. Spiking studies have been carried out for some impurities. The fate and purge of these impurities are described. The limits are acceptable and comply with batches analytical data, applicable guidelines and compendial texts.

The mutagenicity assessment has been performed per ICH M7, using statistical and expert rule-based systems. The genotoxic impurities are in compliance of the guideline ICH M7.

A comprehensive risk assessment was conducted following ICH Q3D guidance. Consideration has been made for other potential elemental impurities: the test results demonstrate that the elemental impurities were consistently below 30% of their respective control thresholds in all sepiapterin batches. Accordingly, no routine testing for elemental impurities is required. The overall risk of elemental impurities for sepiapterin is considered low. This is agreed.

A detailed and adequate risk assessment for potential nitrosamine impurities in sepiapterin active substance and finished product is provided. The risk of introducing N-nitrosamines in sepiapterin through the current manufacturing process is low.

Batch analysis data on several batches of the active substance are provided. The results are within the specifications and consistent from batch to batch.

2.4.2.4. Stability

Stability data of active substance from the proposed manufacturer stored in the intended commercial package under long term conditions (5 ºC) and for up to 6 months under accelerated conditions (25 ºC / 60% RH) according to the ICH guidelines were provided.

All tested parameters were within the specifications.

Photostability testing following the ICH guideline Q1B was performed on one batch. The test results show that the active substance is susceptible to degradation by light.

The stability indicating parameters are well chosen. The assay and purity method (HPLC) are stability-indicating as demonstrated by the forced degradation studies, performed in line with ICH guideline Q1A(R2). Forced degradation studies performed on acid, base, oxidation, metal and thermal stress indicate that sepiapterin active substance can degrade under a variety of stress conditions.

The following parameters were tested: appearance, assay, impurities, chiral purity, water content, crystal form, microbial limits and particle size distribution. The analytical methods used were the same as for release and were stability.

The stability results indicate that the active substance manufactured by the proposed supplier is sufficiently stable. The stability results justify the proposed retest period of 24 months when stored at 5ºC in the proposed container.

2.4.3. Finished Medicinal Product

2.4.3.1. Description of the product and pharmaceutical development

Sepiapterin Oral Powder (Sephience) is an immediate-release solid dosage form available in 250 and 1000 mg strengths. It is presented as yellow to orange powder. The finished product is packaged in heat-sealed aluminium sachets.

The pharmaceutically and clinically relevant physicochemical properties of the active substance were duly identified, being adequately specified and controlled.

All excipients are well known pharmaceutical ingredients and their quality is compliant with Ph. Eur. standards. There are no novel excipients used in the finished product formulation. The list of excipients is included in section 6.1 of the SmPC. Both isomalt (isomaltitol) and mannitol are excipients with known effect and are included in the Annex to the European Commission guideline on 'Excipients in the labelling and package leaflet of medicinal products for human use' with a threshold of "zero" and 10g respectively. The required warning statements according to the Annex are duly mentioned in the package leaflet and SmPC as regards the presence of isomalt.

An active substance - excipient compatibility study was performed under different stressed conditions. The study results under different stressed conditions demonstrate that the active substance has an acceptable stability with the excipients used in the finished product.

Formulation development followed classical approach with the application of design of experiments. The finished product critical quality attributes and process parameters were identified, which is acceptable.

The oral powder is intended to be mixed with water, apple juice, or soft foods prior to administration, to improve the finished product's palatability, which is a known CQA in this type of dosage forms. Sucralose is used as a sweetener, whereas isomalt and mannitol also have sweetening properties. The data on palatability of the finished product is presented and found acceptable.

The proposed dissolution method is well developed and described. The discussion for the suitability of dissolution method was presented with respect to discrimination for the effect of manufacturing process and effect of functional excipients on the drug release pattern. The dissolution method chosen for the finished product has been found to be discriminating on composition and process related changes.

The dissolution specification of "NLT 85% (Q=80%) after 20 minutes" is based on the dissolution results obtained from representative clinical batches. The choice of the manufacturing process is justified. The relevant critical process parameters (CPP) and in-process controls (IPC) were addressed and presented.

Reference to differences in the composition and manufacturing processes of the commercial product and clinical trial formulations are provided and found well justified.

The choice of materials for the container and closure are supported by stability data and compatibility studies.

Compatibility studies with liquid and soft foods are suitable for the proposed use of the finished product.

The feasibility of administering Sephience through enteral tube has been demonstrated using 6 Fr NG and 8Fr NG feeding tubes and a flush volume of 15 mL.

The primary packaging is a heat-sealed laminated aluminium foil sachet: polyethylene terephthalate, white extruded polyethylene (polyester/foil bond), aluminum foil (moisture barrier), and heat-sealed ionomeric resin (adhesive). The material complies with Ph.Eur. and EC requirements. The choice of the container closure system has been validated by stability data and is adequate for the intended use of the product.

2.4.3.2. Manufacture of the product and process controls

The manufacturing process consists of four main steps: pre-blending and screening, roller compaction and milling, final blending and sachet filling. The process is considered to be a standard manufacturing process.

Major steps of the manufacturing process have been validated on commercial scale batches. It has been demonstrated that the manufacturing process is capable of producing the finished product of intended quality in a reproducible manner. The in-process controls are adequate for this type of manufacturing process and pharmaceutical form.

2.4.3.3. Product specification

The finished product release specifications include appropriate tests for this kind of dosage form: appearance (visual), identity (HPLC-UV (DAD), HPLC-UV), assay (HPLC-UV), individual specified identified degradant (HPLC-UV), individual unspecified degradants (HPLC-UV), total degradants (HPLC-UV), uniformity of dosage units (Ph. Eur. – weight variation), dissolution (Ph. Eur.), water content (KF), and microbial limits (Ph. Eur.).

The potential presence of elemental impurities in the finished product has been assessed following a risk-based approach in line with the ICH Q3D Guideline for Elemental Impurities. Based on the risk assessment it can be concluded that it is not necessary to include any elemental impurity controls in the finished product specification. The information on the control of elemental impurities is satisfactory.

A risk assessment concerning the potential presence of nitrosamine impurities in the finished product has been performed (as requested) considering all suspected and actual root causes in line with the "Questions and answers for marketing authorisation holders/applicants on the CHMP Opinion for the Article 5(3) of Regulation (EC) No 726/2004 referral on nitrosamine impurities in human medicinal products" (EMA/409815/2020) and the "Assessment report- Procedure under Article 5(3) of Regulation EC (No) 726/2004- Nitrosamine impurities in human medicinal products" (EMA/369136/2020). Based on the information provided, it is accepted that there is no risk of nitrosamine impurities in the active substance or the related finished product. Therefore, no specific control measures are deemed necessary.

The analytical methods used have been adequately described and appropriately validated in accordance with the ICH guidelines. Satisfactory information regarding the reference standards used for assay and impurities testing has been presented.

Batch analysis results are provided on several batches of various scales confirming the consistency of the manufacturing process and its ability to manufacture to the intended product specification.

The finished product is released on the market based on the above release specifications, through traditional final product release testing.

2.4.3.4. Stability of the product

Stability data from primary stability and clinical study batches of finished product stored under long term conditions (25 ºC / 60% RH), intermediate (30 °C / 75% RH) and accelerated conditions (40 ºC / 75% RH) according to the ICH guidelines were provided. The batches of medicinal product are identical to those proposed for marketing and were packed in the primary packaging proposed for marketing.

Samples were tested for appearance, assay, degradants, dissolution, water content and microbial limits. The analytical procedures used are stability indicating. No significant changes have been observed.

In accordance with EU GMP guidelines[1], any confirmed out-of-specification result, or significant negative trend, should be reported to the Rapporteur and EMA.

In addition, one batch was exposed to light as defined in the ICH Guideline on Photostability Testing of New Drug Substances and Products, demonstrating that the product is sensitive to light

Based on available stability data, the proposed shelf-life of 2 years when stored in the original packaging to protect from light as stated in the SmPC (section 6.3) are acceptable.

2.4.3.5. Adventitious agents

No excipients derived from animal or human origin have been used.

2.4.4. Discussion on chemical, pharmaceutical and biological aspects

Information on development, manufacture and control of the active substance and finished product has been presented in a satisfactory manner. The results of tests carried out indicate consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use.

The applicant has applied QbD principles in the development of the finished product manufacturing process. However, no design spaces were claimed.

2.4.5. Conclusions on the chemical, pharmaceutical and biological aspects

The quality of this product is considered to be acceptable when used in accordance with the conditions defined in the SmPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way.

2.4.6. Recommendations for future quality development

Not applicable.

[1] 6.32 of Vol. 4 Part I of the Rules Governing Medicinal products in the European Union

2.5. Non-clinical aspects

2.5.1. Introduction

Sepiapterin is claimed to be a new molecular entity and an exogenously synthesized, structurally equivalent version of biologically produced endogenous sepiapterin. The mechanism of action of sepiapterin is supported by PTC studies and published literature.

Sepiapterin is being developed for the treatment of Phenylketonuria (PKU), which is characterized by hyperphenylalaninaemia that occurs due to low phenylalanine hydroxylase (PAH) enzyme activity. Low levels of PAH activity can be a result of a defect in the PAH enzyme (ie, PKU) or a lack of its cofactor, BH_4 (i.e., primary BH_4 deficiency) (Smith 2019). In PKU, some variant PAH proteins are mutated (deletion, missense, and nonsense mutations) and misfolded, causing them to proteolytically degrade more rapidly than wild-type PAH. Sepiapterin administration enhances PAH activity, promotes Phe metabolism, and lowers Phe levels in the blood through several distinct and additive mechanisms, which are described below.

Sepiapterin is a natural precursor for the biosynthesis of BH_4 and is rapidly and extensively converted to BH_4 after oral administration (Sawabe 2002, Sawabe 2004, Sawabe 2008, Werner 2011, Smith 2019). The biosynthesis of BH_4 occurs via the pterin salvage pathway, mediated primarily by sepiapterin reductase (SR) and dihydrofolate reductase (DHFR) (Smith 2019), and is potentially assisted by carbonyl reductase (CR) for the first step of reduction (Werner 2011).

Oral administration of sepiapterin has been shown to rapidly produce significant increases in plasma BH_4 in all species evaluated by PTC, including mice, rats, rabbits, dogs, marmoset monkeys, and cynomolgus monkeys (studies are summarized in Module 2.6.4). In mice, rats, marmoset monkeys, and cynomolgus monkeys, the sepiapterin to BH_4 C_{max} and area under the concentration-time curve (AUC) ratios were generally <5%. In dogs and rabbits, the rate of conversion was slower and levels of sepiapterin were higher.

As the key cofactor for aromatic amino acid hydroxylases including PAH, tyrosine hydroxylase (TH), tryptophan hydroxylase (TPH), and nitric oxide synthase (NOS), BH_4 is essential for the metabolism of Phe, tyrosine, tryptophan, and arginine (Werner 2011).

2.5.2. Pharmacology

2.5.2.1. Primary pharmacodynamic studies

As a natural precursor for BH_4 biosynthesis, sepiapterin is rapidly and extensively converted to BH_4 after oral administration via the pterin salvage pathway. This pathway is primarily mediated by sepiapterin reductase (SR) and dihydrofolate reductase (DHFR), with potential assistance from carbonyl reductase (CR) in the initial reduction step.

In vitro studies show that sepiapterin increases the activity of PAH mutant proteins similarly to BH_4. Experiments with COS-7 cells expressing various PAH variants treated with sepiapterin (0, 5, or 20 µM) demonstrated that ten of the fifteen most common PKU genotypes exhibited increased PAH activity, including BH_4-insensitive variants like IVS10-11 G>A. Differential scanning fluorimetry assays under thermal stress indicated that sepiapterin improved the stability of mutant PAH proteins, showcasing its chaperone effect that protects PAH function.

Sepiapterin is more efficiently transported into cells than BH_4, which enters cells through an inefficient two-step process involving oxidation and reduction. While BH_4 uptake can be hindered by the efflux transporter P-glycoprotein (P-gp), sepiapterin is not a substrate for P-gp, allowing for rapid cell entry. Consequently, sepiapterin is more effective than BH_4 at raising intracellular BH_4 levels, as shown in in vitro studies with cell cultures treated with sepiapterin (up to 200 µM).

Oral administration of sepiapterin has been shown to significantly increase plasma BH_4 levels across multiple species, including mice, rats, rabbits, dogs, marmoset monkeys, and cynomolgus monkeys. In mice, rats, marmoset monkeys, and cynomolgus monkeys, the sepiapterin to BH_4 C_{max} and area under the concentration-time curve (AUC) ratios were generally less than 5%. In dogs and rabbits, the rate of conversion was slower, resulting in higher sepiapterin levels.

In various animal models, sepiapterin enhances BH_4-dependent enzymes like nitric oxide synthase (NOS), reducing inflammation, oxidative stress, and endothelial dysfunction by increasing BH_4 levels and recoupling NOS activity. It also improves gastric motility in diabetic rats by restoring neuronal NOS (nNOS) function. Overall, primary pharmacodynamics studies support several interrelated mechanisms of action for sepiapterin, all of which promote PAH enzyme activity, and demonstrate that sepiapterin has biological activity in vivo.

2.5.2.2. Secondary pharmacodynamic studies

The potential risk of off-target interactions was assessed by a non-GLP in vitro study using 94 unique off-targets. There was no inhibition or stimulation greater than 50% observed for any of the receptors studied, indicating that sepiapterin is unlikely to have any significant off-target interactions. Weak or moderate effects were noted with GABA transporter, Lck kinase, COX1 and glycine (strychnine-sensitive antagonism). Furthermore, some publications showed that sepiapterin evaluated in various animal models enhanced the BH_4 dependent enzymes such as NOS in vivo.

2.5.2.3. Safety pharmacology programme

Safety pharmacology studies, following ICH S7A and S7B guidelines, evaluated the effects of sepiapterin on various body systems. In respiratory system studies, sepiapterin showed no adverse effects on respiratory parameters (respiratory rate, tidal volume, and minute volume) at doses up to 1000 mg/kg/day in Sprague Dawley rats. For the central nervous system (CNS), no adverse effects were observed based on functional observation battery evaluations at the same dosage in Sprague Dawley rats. Cardiovascular safety was assessed through both in vitro and in vivo studies. *In vitro* studies indicated that sepiapterin had no effects on the hERG potassium current in HEK293 cells. Sepiapterin had no discernible effects at the highest tested concentration of 30 µM (or approximately 7117 ng/mL, which is 2978× the mean sepiapterin free C_{max} of 2.39 ng/mL at the highest targeted therapeutic dose of 60 mg/kg/day), and tetrahydrobiopterin (BH_4) did not inhibit hERG tail current at the achieved concentration of 866 µM (or approximately 209 µg/mL, which is 908× the mean BH_4 free C_{max} of 230 ng/mL at the highest targeted therapeutic dose of 60 mg/kg/day). *In vivo* studies in male and female marmoset monkeys showed no cardiovascular effects at doses up to 1000 mg/kg/day. Overall, no sepiapterin-related adverse effects were observed in these safety pharmacology studies.

2.5.2.4. Pharmacodynamic drug interactions

No pharmacodynamic drug interaction studies have been conducted to date. However, it is important to consider that sepiapterin enhances BH_4-dependent enzymes such as nitric oxide synthase (NOS). Thus,

potential pharmacodynamic interactions may occur with vasodilatation-inducing drugs, including those administered topically, that affect the metabolism or action of nitric oxide (NO), including conventional NO donors (such as glyceryl trinitrate, isosorbide dinitrate, sodium nitroprusside and molsidomine), phosphodiesterase type 5 (PDE-5) inhibitors and minoxidil, as well as alpha-adrenergic blockers. Such potential for pharmacodynamic interactions has been added to Section 4.5 of the Summary of Product Characteristics (SmPC) and Section 2 of the Package Leaflet.

2.5.3. Pharmacokinetics

Extensive pharmacokinetic (PK) studies were conducted to assess absorption, distribution, metabolism, excretion, and PK drug-drug interaction liabilities of orally administered sepiapterin. Single- and repeat-dose studies evaluating absorption and/or distribution of sepiapterin were conducted in C57Bl/6, CD-1, and CByB6F1 Tg(HRAS)2Jic mice, Sprague Dawley and Long-Evans rats, New Zealand White rabbits, beagle dogs, cynomolgus monkeys, and marmoset monkeys. Metabolism of sepiapterin was evaluated in human intestinal microbiota *in vitro* and in rats and monkeys following a single oral dose of [^{14}C]-sepiapterin. Excretion of sepiapterin was evaluated in single-dose studies in C57Bl/6 mice, Sprague Dawley rats, and cynomolgus monkeys.

Sepiapterin powder has been formulated for oral administration. A formulation for oral suspension in Medisca® Oral Mix™ (flavoured suspending vehicle) was developed and used in nonclinical GLP toxicology studies and initial Phase 1/2 clinical studies. A formulation for oral suspension in Medisca® Ora Plus® was also used during the juvenile toxicity study in rats. A new Phase 3 formulation (mixed in deionized water) was developed; this formulation was used when qualifying drug product impurities in rats. The relative oral bioavailability of these formulations was investigated in male rats, dogs, or monkeys after single administration of sepiapterin: almost similar BH_4 exposures were obtained between various formulations.

The HPLC-MS/MS methods for plasma sepiapterin and BH_4 have been fully validated, ensuring they meet criteria for specificity, sensitivity, accuracy, precision, linearity, and stability. These methods support GLP-compliant toxicology studies, confirming adherence to GLP standards. The LLOQ values are sufficient for accurate pharmacokinetics and toxicokinetics evaluations in mice, rats, rabbits, and marmosets. Sample handling procedures, including the use of K2EDTA tubes and ascorbic acid stabilization, are appropriate and ensure analyte integrity.

The plasma concentration for endogenous sepiapterin and BH_4 was not significant at the pre-dose. Following oral administration, sepiapterin was quickly absorbed in mice, rats (including pregnant female rats), rabbits, dogs, marmoset, and cynomolgus monkeys (T_{max} was generally 2 hours or less). Though measurable, plasma sepiapterin was generally quickly reduced to BQL, and the elimination T1/2 could not be reliably estimated (mice, rats, and marmosets), except in dogs or pregnant female rabbits. Absorbed sepiapterin was rapidly converted to BH_4 in all species studied.

The permeability was studied in vitro: sepiapterin permeability was low in MDCKII-BCRP and Abcb1KO-MDCKII-MDR1 cells and the permeability of BH_4 could not be reliably measured as BH4 was unstable in the assay. Moreover, sepiapterin was a substrate of BCRP and ENT2.

The 28-days repeat-dose study in mice showed that there was little to no increase in BH_4 C_{max} and AUC0-24h values with increasing dose on Days 1 and 28. Systemic exposure (C_{max} and $AUC_{0\text{-}24h}$ values) to BH_4 in mice did not appear to change consistently following repeated administration of sepiapterin. Systemic exposure (C_{max}) to BH_4 was up to approximately 80- to 82-fold greater on Day 1 and up to 35- to 47-fold greater on Day

28 when compared with the systemic exposure of sepiapterin. Following repeated daily dosing in rats, the baseline endogenous sepiapterin concentrations were generally BQL. Following oral administration of sepiapterin in rats, the analyte was generally not measurable in the 14-day, 13-week, and 26-week studies (LLOQ 11 ng/mL), and TK parameters could not be reliably estimated, therefore TK was mainly based on the BH_4 measures. There was no clear accumulation of BH_4 in the 14-day and 26-week studies, while mild accumulation was observed in the 13-week study, suggesting possible saturation kinetics at 300 mg/kg/day. For other studies, BH_4 exposures increased approximately dose proportionally from 30 to 100 mg/kg and less than dose proportionally above 100 mg/kg. In monkeys, plasma sepiapterin was extensively converted to its metabolite BH_4 or quickly eliminated from systemic circulation and dropped to BQL (LLOQ 11 ng/ml) by 8 hours post-dose for the majority of animals. Sepiapterin C_{max} increased less than dose proportionally in the range from 30 to 300 mg/kg. The 13-week toxicity study showed that mean plasma sepiapterin C_{max} and $AUC_{0\text{-}24h}$ were less than 5% of the BH_4 values. There was no accumulation of BH_4, except in the 13-week study where an increase in BH_4 was observed, which was not observed in the 14-day and 9-month (on Day 273) studies. BH_4 exposures increased approximately dose proportionally from 30 to 100 mg/kg and less than dose proportionally above 100 mg/kg. No apparent sex difference in BH_4 exposures was observed in rats and marmoset monkeys.

In pregnant female Sprague Dawley rats and New Zealand White rabbits, sepiapterin was quickly absorbed and extensively converted to BH_4, as in other nonclinical species. Following repeated sepiapterin dosing, BH_4 exposures were lower on GD17 or GD19 compared with GD7. Less than dose-proportional increases in BH_4 exposures were observed, similar to other nonclinical PK studies.

In neonatal rats, endogenous BH_4 was higher on PND4 and PND9 compared to adult rats. Similar to adult rats, the conversion from sepiapterin to BH4 was extensive, and the BH4 to sepiapterin AUC0-24h ratios were between approximately 23.7 and 63.7. Compared to adult rats receiving the same bodyweight-adjusted doses of 30 and 100 mg/kg, BH_4 C_{max} and $AUC_{0\text{-}24h}$ on PND9 were approximately 5-fold and 9-fold higher, respectively, compared to adult rats following 91-day repeated dosing. However, these findings are unique to rats and considered not relevant to humans. As of the data cut-off on 30 June 2024, PTC has collected adequate pharmacokinetic(s) (PK) data to support the age-based dose regimen.

Binding of sepiapterin to human plasma protein was low (around 15.4%) at concentrations from 0.1 to 10 µM in the presence of 0.1% DTT. Binding of BH_4 to human plasma protein was moderate to low at 2, 5, and 15 µM sepiapterin (41.3%, 33.0%, and 24.1% mean plasma protein binding, respectively), when 0.5% β mercaptoethanol was present to stabilize BH_4. The exact impact of stabilising agents on the physiology and structure of each plasma protein and subsequently on the binding of sepiapterin and BH_4 to these plasma proteins is unknown. However, the plasma protein binding studies confirmed that the fraction of sepiapterin and BH_4 bound to plasma protein is low. For these reasons, the plasma protein binding values were not used to derive other physiological parameters.

An approach using total plasma drug concentrations (assuming all drug present in plasma is free drug [100% unbound]) across all species was undertaken in the conduct of interspecies comparisons; therefore, no plasma protein binding studies have been conducted in animal species.

Endogenous sepiapterin was BLQ in plasma (LLOQ 10 ng/mL) and all tissues (LLOQ 50 ng/g) studied in male CD-1 mice. Higher endogenous BH_4 concentration (~ 2.9x) was observed in kidney (291 ng/ g) and the highest concentration (~ 10 x of plasma) was observed in the liver (992 ng/g). Following oral administration, sepiapterin was quickly absorbed, fast distributed to peripheral tissues and converted to BH_4. The peak BH_4 concentrations were observed at 2 hours in plasma and liver and 4 hours in kidney and brain, respectively. The increased BH_4 was quickly eliminated from the plasma, kidney, and liver (T1/2 between 1 and 3 hours). The

absorption rate and elimination rate in brain were slower (T_{max}=4 hours and T1/2 =4.28 hours). BH_4 was well distributed to perfusable tissues such as liver and kidney. In liver, the baseline corrected BH_4 C_{max} and $AUC_{0\text{-}24h}$ were 8.69x and 7.49x of those in plasma. In kidney, the ratio for BH_4 C_{max} and $AUC_{0\text{-}24h}$ to plasma were 4.27x and 4.69x. The bioavailability of BH_4 in brain from orally dosed sepiapterin was much lower. The baseline corrected BH_4 C_{max} and $AUC_{0\text{-}24h}$ in brain were 0.021x and 0.037x of those in plasma.

In LE rats, [14C] sepiapterin-derived radioactivity was widely distributed throughout the body, with most of the tissues showing the highest concentrations at 4 to 8 hours after the dosing, and at 168 hours post-dose, a majority of tissues had concentrations BQL. The endocrine and metabolic/excretory system tissues and the tissues of the gastrointestinal tract contained the highest distribution of [14C] sepiapterin derived radioactivity. Though there was measurable distribution to the tissues in the CNS at select time points, the concentrations in these tissues were lower than those in the tissues in most other tissue systems, and all CNS tissues reached BQL by 48 hours. Distribution of radioactivity to certain melanin-containing tissues, such as the meninges and uveal tract was low and there was no extended retention, indicating that [14C]-sepiapterin-derived radioactivity was most likely not associating with melanin in these tissues. Similarly, the concentrations in the pigmented skin were similar to the concentrations in the non-pigmented skin, and both were BQL at 72 h post-dose.

No standalone studies were performed to assess the milk and placenta transfer of sepiapterin.

The metabolism was studied *in vivo* in SD rats and cynomolgus monkeys although the repeated doses toxicity studies were performed in another species, the marmoset monkey. Sepiapterin was extensively metabolized in rats and monkeys. The major metabolic pathways involved oxidation/dehydrogenation, reduction/hydrogenation, oxidative deamination, dehydration, side chain cleavage, and methylation, alone or in combination. The major metabolic pathways are generally comparable between rats, monkeys, and humans. The metabolism of sepiapterin and BH_4 is not mediated by CYP450 enzymes. Following a single 100 mg/kg oral dose of [^{14}C]-sepiapterin in rats, unchanged sepiapterin was a minor circulating entity, accounting for 0.91% of total radioactivity AUC0-last in plasma. M269/1 and M269/2, possibly BH_4 adducts/artifacts in plasma, were the most abundant circulating metabolites, accounting for 39.4% of total radioactivity AUC0-last in plasma. Following a single 50 mg/kg oral dose of [^{14}C]-sepiapterin in cynomolgus monkeys, unchanged sepiapterin was a minor circulating entity, while M381/1 was the most abundant circulating metabolite, accounting for 16.1% of total radioactivity $AUC_{0\text{-}48h}$ in plasma. In the bile excreta of rats, M221/1 (2'-deoxy sepiapterin), accounting for 0.13% of the dose, was the most abundant metabolite. In the urine, M257/1, accounting for 3.15% of the dose in BDC rats, were the most abundant metabolites. In the faeces, M221/1 (2'-deoxy sepiapterin) was the most abundant metabolite, accounting for 25.1% and 30.0% of the dose in intact rats and BDC rats, respectively. In cynomolgus monkeys, M282/1 and M181/1 (7,8-dihydroxanthopterin) were the two most abundant metabolites in urine, accounting for 1.50% and 1.01% of the dose, respectively. Metabolites M222/1 and M221/1 (2' deoxy sepiapterin) were the most abundant metabolites in faeces, accounting for 9.33% of the dose. The conversion from sepiapterin to BH_4 was slower in dogs and rabbits, and sepiapterin to BH_4 AUC ratios (%) were 24% or higher. Sepiapterin was extensively metabolized in human subjects. M181/1 (7,8-dihydroxanthopterin) and BH_4 were the most abundant metabolites, accounting for 16.4% and 9.2% of the total radioactivity, respectively. All other metabolites were no more than 7% of the total plasma radioactivity. Therefore, there were not major metabolite or unique metabolite. Unchanged sepiapterin was a minor component in urine and was one of the prominent radioactive components in faeces. M282/1 was the most abundant metabolite in urine and accounted for 2.38% of the dose. M224/1 and M238/2 were the most prominent metabolites in faeces, together with sepiapterin, accounting for 10.5% of the dose. Thus, no major metabolite exceeding 10% of total drug-related exposure was detected. Additionally, following a single 4000 mg oral dose of sepiapterin (approximately 60 mg/kg, the highest recommended therapeutic dose) to healthy human subjects (Study PTC923-MD-008-HV), 7 metabolites were identified and all of them were less than 5%

of the total plasma radioactivity (extractable and non-extractable) (Study PTC923-2022-041). As such, no major metabolites have been identified in humans.

The low mass balance recovery of [^{14}C]-sepiapterin-derived radioactivity in human subjects was consistent with observations in monkeys following an oral dose of sepiapterin and may indicate the formation of volatile metabolite(s) as observed in human intestinal microbiota in vitro.

The drug-drug interactions assessment is in clinical part.

2.5.4. Toxicology

2.5.4.1. Single dose toxicity

No single dose toxicity studies have been conducted.

2.5.4.2. Repeat dose toxicity

Repeated dose toxicity studies, in a total of 8 studies, comprised studies in mice, rats and marmoset monkeys with a treatment duration of up to 4, 26 and 39 weeks, respectively. All studies were conducted with administration of sepiapterin once a day by oral gavage; and, except for one 2-week study in rats, were GLP compliant and included toxicokinetic analysis, for both sepiapterin and its metabolite BH_4. Except for a study in mice and the 2-week non-GLP rat study, all studies included recovery periods. The sole study in mice was a study in preparation for a mouse carcinogenicity study. The main repeated dose toxicity studies were, therefore, those conducted in rats and marmoset monkeys.

Rats and marmoset monkeys were selected as the primary species for toxicology studies based on similarities in their PK and metabolic profiles to humans. In addition, reference is also made to a 74% sequence homology between rat and human in the gene for sepiapterin reductase (a key enzyme in the conversion of sepiapterin to BH_4) and a 96% sequence homology in the gene for PAH (phenylalanine hydroxylase).

The GLP-compliant studies in rats comprised studies with 2- 13- and 26-weeks of treatment followed by, respectively, 2- , 4- and 4- weeks recovery periods. The studies in marmoset monkeys had treatment durations of 2- 13- and 39-weeks, followed by, respectively, 2-, 4- and 4-weeks recovery periods.

In terms of tested dose levels, in the studies in rats, the doses tested in the 2-, 13- and 26-week studies were up to 1000, 300 and 200 mg/kg/day, respectively; in marmoset monkeys, the doses tested in the 2-, 13- and 39-week studies were up to 1000, 300 and 300 mg/kg/day, respectively. The rationale for the reduction of the highest tested dose in the 13-week studies, in both species, was based on pharmacokinetic data - little to no increase in BH_4 levels between 300 and 1000 mg/kg/day. Further reduction of the maximum tested dose in the 26-week toxicity study in rats was based on safety data from the 13-week study.

The ratio of systemic exposures to sepiapterin versus its metabolite BH_4 varied between animal species. In rats, sepiapterin was detected in plasma at levels below or very close to the lower limit of quantification. Exposure to sepiapterin was markedly lower than that to BH_4 in marmoset monkeys (and also in mice). For comparison, in humans, systemic exposure to sepiapterin is also markedly lower than that to metabolite BH_4.

Systemic exposure to sepiapterin (in marmoset monkeys, and also in mice), generally increased with dose but less than dose proportionally. As for sepiapterin, systemic exposure to BH_4 generally increased less than proportionally to the increase in dose level. There was no clear sex difference in exposure to BH_4 and no marked

or consistent accumulation with repeated dose administration. BH_4 was detected in control animals, which is not unexpected as BH_4 is an endogenous substance.

Adverse effects identified in the studies in rats were limited to effects in the kidneys. Renal tubular degeneration/regeneration with deposition of crystals in the collecting tubules and ducts in the kidney were noted at 300 and ≥100 mg/kg/day in the 13-and 26-week studies, respectively. In the 26-weeks studies, there was also an associated minimal to mild tubular epithelial hyperplasia (simple hyperplasia) in the papilla which was attributed to a physical reactive response to the presence of crystals and not preneoplastic, interstitial inflammation (minimal to moderate), interstitial fibrosis (minimal to mild), and tubular dilatation (minimal to mild) and a low incidence of crystals (minimal) in the renal pelvis, which was considered to support the induction of crystal nephropathy by sepiapterin administration. At the NOAEL for the 13- and 26- week studies, systemic exposures (AUC) to BH_4 in males/females were approximately 6-/7- and 2-/2-fold higher than that expected in humans.

No effects considered to be adverse were identified in the studies conducted in marmoset monkeys, in which the maximum tested doses in the 2- 13 and 39-week studies corresponded to systemic exposures (AUC) to BH_4 in males/females of approximately 6-/10-, 2-/3- and 8-/5- fold, respectively, higher than that expected in humans.

All margins of exposure were determined based on total concentrations (plasma protein bound and unbound), as binding to animal plasma proteins has not been tested.

Data from the different studies in rats and marmoset monkeys are further detailed below:

In rats

In the pivotal 14-day study, sepiapterin was administrated at 100, 300, 1000 mg/kg/day by oral route (gavage) in SD rats. The dose levels were determined on a preliminary 14-day non-pivotal study (only female tested, limited histopathology analysis) and a 2-day preliminary study (not submitted). In the non-pivotal 14-day study, sepiapterin was well tolerated and no findings were observed up to the highest tested dose (240 mg/kg), the second preliminary study administered a high dose of 600 mg/kg/day for 2 days and was well tolerated with no clinical observations. Sepiapterin was rapidly converted to its metabolite BH_4, therefore, BH_4 measurement as proof of exposure could be considered adequate. In the pivotal 14-day study, central nervous system behaviour and respiratory function were also assessed. A 14-day recovery period was added for the control group and the high dose group. Sepiapterin up to 1000 mg/kg/day was well tolerated and no findings were observed in rats. The highest dose could be considered as a NOAEL as indicated by the Applicant.

In the 13-week study in rats, sepiapterin was tested at dose levels of 30, 100 and 300 mg/kg/day. A recovery period of 28-day was added for control and high dose groups. The maximum dose of 300 mg/kg/day was selected since BH_4 exposures at this dose is similar than those achieved at 1000 mg/kg/day (observed in 14-day study) and represented a 10-fold the clinical exposure obtained at the time in Phase 1 clinical trial (healthy volunteers dosed at 80 mg/kg). Test-related findings were detected in GI tract and kidneys. Findings in the GI tract consisted of soft material and/or creamy material in the lumen of the stomach, duodenum, jejunum, ileum, and/or cecum at all doses including control, with a dose-related, slightly increased incidence at 100 and 300 mg/kg/day. These macroscopic findings had no microscopic correlates and not observed at the end of the recovery period. In addition, these findings were not observed in 26-week study. The only target organ identified was the kidney. Findings in the kidney included mottled/pale areas, tubular degeneration with or without crystal deposits, increased kidney weights, as well as increased serum urea and creatinine levels at 300 mg/kg/day. Tubular degeneration was partially reversible and elevated serum urea levels resolved, while

increased kidney weights and elevated serum creatinine levels persisted by the end of the 4-week recovery period. The NOAEL was determined to be the mid-dose (100 mg/kg/day).

In the 26-week in rats, sepiapterin was tested at 30, 100 and 200 mg/kg/day. A recovery period of 28-day was added except for the low dose groups. The dose selection could be considered acceptable. Kidney was also identified as a target organ. Adverse renal findings were characterized by areas of moderate to marked tubular degeneration and regeneration. In addition, the deposition of tubular crystals within the distal papillary collecting tubules and ducts in the kidney, moderate interstitial inflammation, and minimal to mild renal fibrosis were observed. Elevated serum urea and creatinine levels were noted at 200 mg/kg/day in individual males with adverse renal findings. The deposition of crystals was associated with a minimal to mild tubular epithelial cell hyperplasia (simple hyperplasia). At the end of 4-week recovery period, moderate tubular degeneration, moderate interstitial inflammation, and mild interstitial fibrosis were noted in only one 100 mg/kg/day female. Renal tubular hyperplasia (simple hyperplasia) was not observed after this recovery period, where crystals were no longer present. Adverse microscopic findings were also observed in urinary bladder (one male at 200 mg/kg). Multiple calculi were observed accompanied by distention of the urinary bladder, ureters and urinary pelvis, and histologically associated with adaptive changes in the urothelium (hyperplasia/metaplasia) and partial obstruction of urine outflow and pyelonephritis. These findings in urinary bladder were not observed in recovery period. Finally, a test-related increased incidence/severity of hepatocellular centrilobular vacuolation was observed in male at 200 mg/kg/day and was not considered adverse due to the low severity of this finding and its absence in recovery group. The NOEL dose was set at the lowest dose of 30 mg/kg/day, with a safety margin based on the BH_4 exposure of around 1.5. The LOAEL was determined at 100 mg/kg/day, the renal adverse findings appear at 3.2-fold (in female rat) and 4.7- fold (in male rat) the clinical BH_4 at the maximum recommended human dose.

In marmoset monkeys

In NHP program, a 14-day study in marmoset was performed (GLP-study) with a 14-day recovery period. Sepiapterin was dosed at 100, 300 and 1000 mg/kg/day. One female at 100 mg/kg/day died at D7 (meningoencephalitis, not considered as treatment related by the Applicant). NOAEL was set at the highest dose since the Applicant considered the brain and kidney findings were not treatment related. After pathology report review, kidney findings observed in females at 1000 mg/kg/day could be considered as treatment related and only partially recovered at the end of recovery period time. In addition, kidney was identified as a target organ in the rat studies. Therefore, the NOAEL need be set at 300 mg/kg/day.

A 13-week study was performed in monkeys and a 28-day recovery period was added. Sepiapterin was dosed at 30, 100 and 300 mg/kg/day. Three animals were found dead during the course of this study but were not considered as treatment related by the Applicant since all had findings that were considered spontaneous findings for marmosets. No test article-related gross or microscopic findings were observed. The Applicant has set the NOAEL at the highest tested dose 300 mg/kg/day.

A 39-week study was performed in marmoset monkeys with a 4-week recovery period. Sepiapterin was administrated at 30, 100, 300 mg/kg/day by oral route. During this study, there were 12 early deaths (unscheduled euthanasia and animals found dead). Animals who died before D30 were replaced to maintain a sufficient number of animals in each group in the study. No unscheduled deaths were considered related to administration of sepiapterin by the Applicant due to the absence of consistent gross and/or microscopic changes and lack of a pattern.

2.5.4.3. Genotoxicity

The genotoxic potential of sepiapterin was investigated in a standard test battery.

Sepiapterin was tested in a bacterial reverse mutation assay using 5 strains of *Salmonella typhimurium* (*S. typhimurium*) TA1535, TA1537, TA98, TA100, and TA102 with and without S9 mix. Sepiapterin did not show any mutagenic activity either in the presence or absence of a rat liver metabolizing system.

Sepiapterin was also evaluated for the potential to induce chromosome aberrations in cultured human lymphocytes in the presence and absence of S9 mix.

The selected dose levels were 0.031, 0.063, 0.125, 0.25, 0.5, and 1 mM for the 3- and 20-hour treatments without S9 mix and 0.031, 0.063, 0.125, 0.25, 0.5, 0.75, and 1 mM for the treatment with S9 mix. Following the 3-hour treatment, a slight increase in the frequency of cells with structural chromosomal aberration was noted at 0.5 and 1 mM. At these dose levels, the frequencies were slightly above the vehicle control historical range (4.3% and 3.3%, respectively, *versus* 0.0% 2.0% for the historical data), and a statistical significance relative to the vehicle control was observed ($p<0.05$). In the absence of a dose-response relationship, these results met the criteria for neither a positive nor a negative response and were considered to be an equivocal response.

Following the 20-hour treatment, a dose-related increase in the frequency of cells with structural chromosomal aberration was noted. At 0.25, 0.5, and 1 mM, the frequencies ($p<0.001$ at 0.5 and 1 mM relative to vehicle) were above the vehicle control historical range (5.3%, 22.0%, and 35.0% versus 0.0% to 3.5% for the historical data). These results met the criteria for a positive response.

In the presence of the S9 mix, slight decreases in the MI were observed at dose levels of 0.063, 0.125, 0.5, and 1 mM, without any clear evidence of a dose-response relationship (25% to 37% decrease in the MI). Because no consistent evidence of cytotoxicity was present, the dose levels selected for metaphase analysis were 0.25, 0.5, and 1 mM. At the dose level of 1 mM, the frequency of cells with structural chromosomal aberration (3.0%) was slightly above the vehicle control (2.7%) and the vehicle control historical range (0.0% to 2.5%). Nevertheless, the vehicle control was also slightly above the vehicle control historical range. In the absence of statistical significance relative to vehicle control and in the absence of a dose response relationship, this minimal increase is most probably not biologically relevant. No other increase in the frequency of cells with structural chromosomal aberration was noted. According to the Applicant, these results were considered to meet the criteria for a negative response.

In conclusion, sepiapterin did not induce chromosome aberrations in cultured human lymphocytes in the presence of rat liver S9. Notwithstanding, without metabolic activation, sepiapterin induced structural chromosomal aberrations following 20-hour continuous treatment while the conclusion remained equivocal following the short (3-hour) treatment period.

Because of this *in vitro* signal was observed in the chromosomal aberration assay, two *in vivo* mammalian toxicity evaluations (micronucleus assay and comet assay) were conducted in male Sprague Dawley rats administered sepiapterin as advised in ICH S2(R1).

In the micronucleus assay, sepiapterin was dosed at 100, 300, 1000 and 2000 mg/kg/day for 3 days, and no increase in the frequency of micronucleated polychromatic erythrocytes (MN-PCE) was observed for male rats administered sepiapterin at any dose level, and there was no evidence of a dose response. The lack of an effect on polychromatic erythrocyte (PCE) frequency indicates there was no bone marrow cytotoxicity.

However, in Comet assay associated to micronucleus assay, a small but statistically positive increase in DNA damage in liver was observed in rats administered 1000 mg/kg/day sepiapterin compared to the vehicle control group. Exposure of BH_4 at 2000 mg/kg/day is lower than at 1000 mg/kg/day. Therefore, exposure at 1000 mg/kg is therefore considered the highest exposure in the study. However, this finding could be considered not biologically relevant since statistically significant DNA damage values at 1000 mg/kg/day were below the mean ±standard error of the mean (±SEM) of the Testing Facility historical control data.

As supported by S2(R1), negative results in two appropriate in vivo assays, with the demonstration of exposure to both sepiapterin and the metabolite BH_4, may be considered sufficient to demonstrate absence of genotoxic risk.

2.5.4.4. Carcinogenicity

In January 2021, as part of Scientific Advice (EMEA/H/SA/4715/1/2020/III), the Committee for Medicinal Products for Human Use (CHMP) agreed that further experiments would be of no value in assessing the long-term oncogenic potential in man. As advised by the CHMP, a comprehensive Weight of Evidence (WOE) assessment has been completed.

The following key points are of relevance for the WOE assessment:

- Sepiapterin and BH_4 both naturally occurring endogenous cofactors;
- The nonclinical and clinical data show rapid conversion of sepiapterin to BH_4 following oral administration and thus minimal systemic exposure to sepiapterin;
- The lack of carcinogenic potential of sapropterin (synthetic BH_4) is well established and based on the mechanism of action and cellular pathways for sepiapterin and BH_4, there are no known pathways involved with any human carcinogenic risk (Friedrich and Olejniczak 2011, Joppi 2013).
- Carcinogenicity studies have been performed with sapropterin. In the 2-year rat carcinogenicity studies with sapropterin, there were no preneoplastic or neoplastic findings at 250 mg/kg/day. Following single oral administration of sapropterin in rats, the systemic exposure (AUC) was reported to be 4571 h•ng/mL at 100 mg/kg/day (KUVAN Pharmacology Review). The exposure at 100 mg/kg/day sapropterin in rats is 1.5× the BH_4 exposure in PKU patients dosed with sepiapterin (PTC923 MD 003 PKU). Considering that the NOAEL was 250 mg/kg/day in the 2-year rat carcinogenicity study with sapropterin, BH_4 exposures in this carcinogenicity study exceed the exposures of BH_4 observed in PKU patients dosed with sepiapterin. A 2-year mouse carcinogenicity study with sapropterin also indicated no potential to induce neoplastic or hyperplastic lesions (KUVAN EPAR 2008)
- In the postmarketing surveillance study in 85 Japanese sapropterin-responsive PKU patients administered sapropterin with a treatment duration between 0.2 to 17.4 years and where treatment started at age <4 years in 50.6%, there was no indication of any cancer-related adverse events. Furthermore, examination of the data collected in the long-term sapropterin registry (PKUDOS), which presents safety data for 7 years of sapropterin exposure, showed no indication of any cancer-related adverse events/reactions .
- Sepiapterin may be considered to be not genotoxic (pending further clarifications).
- There were no sepiapterin-related preneoplastic or neoplastic changes indicative of any on- or off-target potential of carcinogenic concern in the chronic toxicity (6-month rat and 9-month marmoset monkey) studies with sepiapterin.

- Sepiapterin caused no disturbance of endocrine and reproductive organs or immune modulation in the repeat-dose toxicity or reproductive and developmental studies. Sepiapterin caused no off-target toxicity.
- No safety concerns have been reported in the completed and ongoing clinical trials with sepiapterin.

Notwithstanding all the issues above, some other aspects merit discussion that should be included as part of the WOE approach. In 26-week rat study, several hyperplasia was noticed. The Applicant discussed hyperplasia observed in kidney and concluded this hyperplasia of the papilla in kidney is not considered as pre-neoplastic change. The discussion presented could be considered acceptable. The Applicant discussed also hyperplasia observed in pituitary glands and concluded that this hyperplasia is most likely incidental and spontaneous in this age of laboratory SD rats and unlikely related to treatment with sepiapterin, the rationale is also acceptable. Other hyperplasias were observed but were only present in one treated animal or in one control and one treated animal. No increased incidence of hyperplasias were noted in treated group compared in control group. However, hyperplasias were observed at terminal necropsy in pancreatic islets in 6/15 male rats in control group (1 focal and 5 multifocal) and 3/15 male rat in high dose group 200 mg/kg/day, at the recovery necropsy, no hyperplasias were observed in control group and hyperplasia was identified in 2 male rats in high dose group. The Applicant has justified that these findings were within the ranges noted in the Testing Facility HCD; therefore, it was not considered a significant finding. The historical control values were provided. The Applicant has clarified that no hyperplasia was observed in long-term studies.

Additionally, results of 26-week carcinogenicity study in transgenic mice were submitted.

Sepiapterin was administrated for 26 weeks in Tg mice at dose of 30, 100 and 300 mg/kg/day in males and 100, 300 and 1000 mg/kg/day in females; the dose selection is not explained in the report. However, the exposure to BH4 in the high dose group is female is 16-fold the clinical exposure to BH4 at the MHRD and 11-fold for the male group. Therefore, the dose selection could be considered acceptable. The survival rates are acceptable (>90%). As indicated in the report, there was a slight increase in incidence of bronchioloalveolar adenoma in the lung of females at 1000 mg/kg/day sepiapterin when compared to vehicle control and water control. This was accompanied by a slight increase in incidence of bronchioloalveolar hyperplasia (non-neoplastic proliferative finding) at 300 mg/kg/day (2/25, 8%) and 1000 mg/kg/day (2/25, 8%) when compared to vehicle control (0/25, 0%) and water control (1/25, 4%). However, the incidence of bronchioloalveolar adenoma (2/25, 8%) was within that of the Testing Facility historic control data (0% to 16%) and the incidence of bronchioloalveolar hyperplasia (2/25, 8%) was within that of the Testing Facility historic control data (0% to 13.04%). In addition, the incidence of bronchioloalveolar adenoma, bronchioloalveolar carcinoma, and the tumour combination of bronchioloalveolar adenoma/carcinoma was not statistically significant. Therefore, this finding was not considered related to sepiapterin administration. In addition, the incidence of benign thymoma (2/25, 8%) in the thymus at 100 mg/kg/day was increased compared to vehicle control and water control. However, this incidence was within that of the testing facility historic control data (0% to 8.70%). This was also not dose responsive (present at low dose only) and, therefore, this was not considered related to sepiapterin administration. Historical control data was indeed provided and consistent with the conclusion. Therefore, it could be concluded that no carcinogenic effect related to sepiapterin administration was observed at any dose level in males or females in this study. This result reinforces the WOE approach submitted by the Applicant which is suggested that the carcinogenicity risk with sepiapterin is low.

2.5.4.5. Reproductive and developmental toxicity

Reproductive toxicity comprised studies on fertility and early embryonic development (rat), embryo-foetal development (rat and rabbit), pre- post-natal development (rat) and juvenile animal studies (rat).

Fertility and early embryonic development:

Studies on fertility and early embryonic development comprised one study in rats. This was GLP-compliant and had administration of sepiapterin once a day by oral gavage to males and females at doses levels up to 300 mg/kg/day.

The study revealed no adverse effects (parental general toxicity or reproductive toxicity). The study has not included a toxicokinetic analysis. Based on extrapolation of toxicokinetic data from repeated dose toxicity studies in rats, systemic exposures (AUC) to BH_4 at the maximum tested dose are estimated to have been approximately 7-8-fold higher than that expected in humans.

Embryo-foetal development:

Studies on embryo-foetal development comprised dose-range finding (non-GLP) and pivotal (GLP) studies in rats and rabbits. In all studies, sepiapterin was administered once a day by oral gavage at dose levels of 100, 300, and 1000 mg/kg/day. All studies included analysis of maternal plasma for toxicokinetics.

No effects on embryo-foetal development were considered to have been observed. In terms of general maternal toxicity, effects observed, which were not considered to be adverse, were limited to reduction in body weight and food consumption in rabbits.

At the maximum tested dose in the pivotal studies in rats and rabbits, systemic exposures (AUC) to BH_4 were 9- and 6-fold higher, respectively, than that expected in humans.

Regarding toxicokinetic data, it is also of note that, in rabbits, systemic exposure to sepiapterin was lower than that observed for its metabolite BH_4, but the difference was less marked than that observed for marmoset monkeys.

Pre- post-natal development:

Studies on pre- post-natal development comprised one study in rats. This study was GLP compliant and had sepiapterin administered once a day by oral gavage at dose levels of 30, 100 and 300 mg/kg/day.

The study revealed no adverse effects (parental general toxicity or reproductive toxicity). Although it has not included a toxicokinetic analysis, based on extrapolation of toxicokinetic data from repeated dose toxicity studies in rats, systemic exposure (AUC) to BH_4 at the maximum tested dose is estimated to have been approximately 7-fold higher than that expected in humans.

Juvenile animal studies:

Studies with direct administration to juvenile animals comprised two studies in rats, both GLP compliant, with administration of sepiapterin once a day by oral gavage, and including toxicokinetic analysis (sepiapterin and BH_4). One of studies was a pharmacokinetic study with a treatment duration of 6 days, from PND4 to PND9, and tested dose levels of 10, 30 and 100 mg/kg/day. The second study aimed at determining potential toxicity of sepiapterin and toxicokinetics. Animals were treated for 10 weeks from PND4 to PND70 with lower dose levels administered for PND4 to PND29, compared to the subsequent treatment days - 5/30, 10/100 and 30/300 mg/kg/day-, so to allow for exposures to be consistent throughout the dosing period. The treatment phase was followed by a 4-week recovery period. Study assessments included mortality, clinical observations, body weight and body weight gain, food consumption, sexual maturation, clinical pathology (haematology, coagulation, clinical chemistry, and urinalysis), behavioural assessments (motor activity, acoustic startle habituation, and Morris water maze), organ weights, bone length and densitometry, and macroscopic and microscopic examinations, sperm motility, concentration, and morphology, and oestrous cycles.

In terms of pharmacokinetics, the studies revealed that, for identical dose levels of sepiapterin, systemic exposures (AUC) to BH_4 were higher in neonatal rats compared to adults. However, as clarified by the Applicant, these findings are unique to rats and considered not relevant to humans (see section on pharmacokinetics).

No toxicities have been observed in the studies. At the maximum tested doses in the 10-week study (30/300 mg/kg/day), systemic exposure (AUC) to "composite" BH_4 in males/females on PND4, 29 and 70 were approximately 7-/13-, 9-/10- and 4-/3- fold, respectively, higher than exposure to BH_4 in humans.

All margins of exposure were determined based on total concentrations (plasma protein bound and unbound), as binding to animal plasma proteins has not been tested.

2.5.4.6. Toxicokinetic data

The ratio of systemic exposures to sepiapterin *versus* its metabolite BH_4 varied between animal species. In rats, sepiapterin was detected in plasma at levels below or very close to the lower limit of quantification. Exposure to sepiapterin was markedly lower than that to BH_4 in marmoset monkeys and also in mice. In rabbits, exposure to sepiapterin was still lower compared to BH_4, but less markedly (up to 8-fold lower based on AUC). Systemic exposure to sepiapterin was also markedly lower than that of metabolite BH_4 in humans.

Systemic exposure to sepiapterin (in marmoset monkeys, rabbits and also in mice), generally increased with dose but less than dose proportional. As for sepiapterin, systemic exposure to BH_4 generally increased less than proportionally to the increase in dose level. There was no clear sex difference in exposure to BH_4 and no marked or consistent accumulation with repeated dose administration.

All data refer to total concentrations (plasma protein bound and unbound), as binding to animal plasma proteins has not been tested.

2.5.4.7. Local Tolerance

Sepiapterin is administered orally, so no local tolerance studies are considered necessary.

2.5.4.8. Other toxicity studies

A thorough review of relevant data outlining potential drug dependence of sepiapterin was conducted by the Applicant and described in the dossier. Specifically, effects of sepiapterin and BH_4 on neurotransmitters associated with drug dependence (e.g., dopamine and serotonin), nonclinical safety data from sepiapterin, activity of sepiapterin at possible secondary neurotransmitter targets related to drug dependence, relevant literature, chemistry and manufacturing, and publicly available drug class-relevant data were reviewed and summarized. The most relevant points were collated above and illustrate the conclusion that the potential for development of sepiapterin dependence appears to be extremely low. Existing non-clinical and clinical evidence is supportive of these conclusions, along with the experience accumulated with sapropterin (a drug that like sepiapterin is a BH_4 modulator) approved in the EU in 2008. Based on the information available, no specific toxicological issues are of concern regarding the development of dependence.

Process-related impurities were observed in sepiapterin DS and were satisfactorily qualified in toxicology studies. In addition, potential degradants in sepiapterin DP were also qualified in toxicology studies. The safety

margins for impurities, degradants, and enantiomer in the DS and DP based on specifications and qualified levels in the 28-day rat studies PTC923-2021-007 and PTC923-2023-044 are summarized as follows:

Table 6: Summary of safety margins for impurities, degradants, and enantiomer in the DS and DP based on specifications and qualified levels in the 28-day rat studies PTC923-2021-007 and PTC923-2023-044

DS Impurity				
Impurity Name		**Specification (Area %)**	**Qualified Level (Area %)**	**Safety Margin**
RRT0.28	CC-49	NMT 0.15	0.97	6.5
RRT0.55	CC-77	NMT 0.5	0.68	1.4
RRT0.95	CC-06	NMT 1.5[a]	1.7	1.1
RRT1.07	CC-80	NMT 0.3	0.41	1.1
RRT1.13	CC-10	NMT 0.14	0.14	1.0
RRT1.16	CC-11	NMT 2.0	2.2	1.1
DP Degradant				
Impurity Name		**Specification (Area %)**	**Qualified Level (Area %)**	**Safety Margin**
RRT0.52	CC-75	NMT 0.38	0.61	1.3
RRT0.61	CC-66	NMT 0.25	0.76	2.4
RRT0.66	CC-77	NMT 0.32	0.95	3.0
RRT0.75	CC-49	NMT 0.28	0.62	2.2
RRT0.85	CC-06	NMT 1.28[a]	2.3	1.4
Enantiomer				
Enantiomer		**Specification (Area %)**	**Qualified Level (Area %)**	**Safety Margin**
CC-18 (R-SP)		NMT 1.0	1.5	1.5

Abbreviations: DP, drug product; DS, drug substance; NMT, not more than; RRT, relative retention time; R-SP, R enantiomer of sepiapterin

[a] For drug substance, the specification will be set at 1.5%. For drug product, the specification will be set at 2.0% or equivalent.

Based on the 28-day rat toxicology studies, the qualified levels of all of the impurities are at or above the specifications proposed in Module 3. The safety and quality data indicate there is no apparent mutagenic risk for humans associated with sepiapterin.

Study PTC923-2021-010 was a GLP-compliant study designed to evaluate the phototoxic potential of sepiapterin as measured by the relative reduction in viability of BALB/c 3T3 mouse fibroblasts exposed to sepiapterin and ultraviolet radiation (+UVR), as compared with the viability of fibroblasts exposed to sepiapterin in the absence of ultraviolet radiation (-UVR). Promethazine was used as the positive control. The phototoxic evaluation of sepiapterin was assessed using photoirritancy factor (PIF) and mean photo effect (MPE) endpoints. Up to the highest concentration tested (111 µg/mL), sepiapterin in 1% DMSO in DPBS did not demonstrate phototoxic potential in the neutral red uptake phototoxicity assay.

An age-related risk assessment of the excipients was performed for paediatric and adult patients. Based on the toxicity information from the preclinical and clinical studies described in the published literature and relevant databases (e.g., European Paediatric Formulation Initiative [EuPFI] Safety and Toxicity for Paediatrics [STEP]

database and US FDA Inactive Ingredient Guide [IIG]), the total daily amount of each excipient administered to paediatric and adult patients is not expected to pose a safety risk to children and adults taking the maximum strength of sepiapterin per day.

Isomalt is a sugar substitute, a type of sugar alcohol used primarily for its sugar-like physical properties (FAO/WHO 1985, McNutt and Sentko 2003). Complete hydrolysis of isomalt yields glucose (50%), sorbitol (25%), and mannitol (25%). Isomalt is a food additive and is generally regarded as safe (GRAS) by the US FDA. The World Health Organization (WHO) considered it unnecessary to set a numerical acceptable daily intake (ADI) for isomalt. The low toxicity of isomalt shown in toxicological studies supports this conclusion. The feeding of isomalt at levels up to 10% in the diet of rats (corresponding to about 5000 mg/kg body weight), starting with exposure in utero and continuing for 1 year, did not induce any toxic effects. However, laxative effects in humans were noted at high intake of 20 to 30 g per day (Lee A 2002). The estimated maximum daily exposure (mg/day) of isomalt that a patient would receive is 5760 mg/day or 96 mg/kg/day for patients with an average 60-kg body weight. This is 3.5× lower than the dose which may be related to laxative effects in humans. This is also 50× below the NOAEL in a 1-year repeat toxicity study in rats. Therefore, the maximum potential exposure to isomalt in this case is not expected to pose a safety risk to patients.

2.5.5. Ecotoxicity/environmental risk assessment

Synthetic sepiapterin (formerly PTC923, CNSA-001) is a new chemical entity, that is an exogenously synthesised, structurally equivalent version of biologically produced endogenous sepiapterin, a naturally occurring precursor for tetrahydrobiopterin (BH_4).

The Applicant has provided an environmental risk assessment (ERA) Phase I of Sephience (sepiapterin) oral powder 250 mg and 1000 mg, by the Guideline on the Environmental Risk Assessment of Medicinal Products for Human Use (CHMP/SWP/4447/00, 2006) (Table 7 below).

Table 7: Summary of main study results

Substance (INN/Invented Name): Sepiapterin			
CAS-number (if available):			
PBT screening		Result	**Conclusion**
Bioaccumulation potential- log K_{ow}		-1.43	Potential PBT: N
PBT-assessment			
Parameter	**Result relevant for conclusion**		**Conclusion**
Bioaccumulation	log K_{ow}	1.43	not B
	BCF		B/not B
Toxicity	NOEC or CMR		T/not T
PBT-statement:			
Phase I			
Calculation	**Value**	**Unit**	**Conclusion**
PEC $_{surfacewater}$, refined	0.28	μg/L	> 0.01 threshold

			Y
Other concerns (e.g., chemical class)			Y/N

Phase I:

The Applicant has performed recently a study according to OECD 107 and has submitted the report in Annex to the module 1.6 (GLP study). The determined value indicated that for sepiapterin, log Kow is below the Log Kow trigger value of 4.5. it is agreed that a PBT assessment is not required.

The Applicant determined refined PECsurfacewater for Sepiapterin calculated with a refined Fpen obtained with prevalence of the pathology PKU. Since the obtained value of 0.28 µg/L is superior to the action limit defined in the *"Guideline on the Environmental Risk Assessment of Medicinal Products for Human Use'* (CHMP/SWP/4447/00, 2006) of 0.01 µg/L. Therefore, a Phase II environmental effect analysis and risk assessment is required. The Applicant has decided to not perform Phase II studies on the rationale that sepiapterin is a naturally occurring, endogenous substance that is present in humans and in other animals, and is unlikely to endanger the environment, according to the Guideline EMEA/CHMP/SWP/4447/00 Rev1, 2006. Nevertheless, it is expected to be present in the environment and no existing data on background levels in the environmental were identified. The rationale could be considered accepted.

Precautionary and safety measures taken to reduce any risk to the environment by including the general statement on the SmPC and PL were applied, according to the Guideline on the environmental risk assessment of medicinal products for human use EMEA/CHMP/SWP/4447/00, 2006.

2.5.6. Discussion on non-clinical aspects

The pharmacology data support sepiapterin as a promising treatment for phenylketonuria (PKU). Sepiapterin enhances PAH activity, promotes phenylalanine metabolism, and lowers blood phenylalanine levels through multiple mechanisms. It is efficiently converted to BH_4, improves stability of mutant PAH proteins, and demonstrates significant biological activity in various animal models. Safety studies indicate no adverse effects on respiratory, central nervous, or cardiovascular systems, underscoring its potential as a safe and effective therapeutic option for PKU. Potential interactions with other drugs, particularly those leading to hypotension such as PDE5 inhibitors, NO donors, and alpha receptor blockers, have been added to Section 4.5 of the Summary of Product Characteristics (SmPC) and Section 2 of the Patient Information Leaflet.

Sepiapterin demonstrates favourable pharmacokinetic properties, with rapid absorption, widespread distribution, and efficient metabolism to BH_4. Its minimal interaction with major drug-metabolizing enzymes and transporters supports its potential as a safe and effective treatment for HPA in PKU patients. Compared to adult rats receiving the same body weight-adjusted dose of 30 and 100 mg/kg, BH_4 C_{max} and $AUC_{0\text{-}24h}$ on PND9 were approximately 5× and 9× that of adult rats following 91-day repeated dosing. Given that sepiapterin is proposed for treating hyperphenylalaninaemia (HPA) in both adult and paediatric patients with phenylketonuria (PKU), the Applicant was requested to clarify the pharmacokinetic factors contributing to these age-related differences and provide a rationale for the dose adaptation strategy according to age. The Applicant clarified that the age-related differences in BH_4 exposure in rats are not relevant to humans. The proposed age-tiered, body weight-adjusted dosing strategy, based on a population pharmacokinetic model and clinical data, ensures appropriate pharmacokinetic exposure across all age groups. The strategy has demonstrated efficacy and safety in both adult and paediatric PKU patients, justifying the approach.

The non-clinical program was performed according to the ICH M3 guideline.

No single dose toxicity studies have been conducted. This is acceptable. Although acute toxicity has not been identified in other toxicological studies, in short-term (2 weeks) repeated dose toxicity studies in rats and marmoset monkeys, sepiapterin was tested at doses up to 1000 mg/kg/day with corresponding margins of exposure reaching the 10-fold exposure in humans; in a 1-month repeated dose toxicity study in mice, it was tested at doses up to 4000 mg/kg/day, a maximum feasible dose based on the maximum feasible concentration.

Repeated dose toxicity studies, in a total of 8 studies, comprised studies in mice, rats and marmoset monkeys with a treatment duration of up to 4, 26 and 39 weeks, respectively. All studies were conducted with administration of sepiapterin once a day by oral gavage; and, except for one 2-week study in rats, were GLP compliant and included toxicokinetic analysis, for both sepiapterin and its metabolite BH_4. Except for a study in mice and the 2-week non-GLP rat study, all studies included recovery periods. The sole study in mice was a study in preparation for a mouse carcinogenicity study. The main repeated dose toxicity studies were, therefore, those conducted in rats and marmoset monkeys.

Rats and marmoset monkeys were selected as the primary species for toxicology studies based on similarities in their PK and metabolic profiles to humans. In addition, reference is also made to a 74% sequence homology between rat and human in the gene for sepiapterin reductase (a key enzyme in the conversion of sepiapterin to BH_4) and a 96% sequence homology in the gene for PAH (phenylalanine hydroxylase). Sepiapterin has a dual chaperone activity. Sepiapterin and BH_4 presented two activities: activity of BH_4 as a cofactor for the PAH enzyme, which is relevant for toxicology studies in rats and marmosets with wildtype PAH, and an chaperone mechanism of action of at least BH_4 (questionable for sepiapterin given its rapid conversion and consequently limited exposure) which is only relevant in the context of mutant PAH variants; it is not relevant to toxicology studies, in which rats and marmosets have wildtype PAH gene/protein structure resulting in functional PAH enzyme. Therefore, only the effect of BH_4 as a cofactor for PAH enzyme, a mechanism shared by sepiapterin and sapropterin, is the key mode of action relevant for toxicological and carcinogenicity assessment. The healthy animals could therefore be considered as partially relevant. In addition, endogenous levels of BH_4 in animals are far higher than those measured in humans.

Adverse effects identified in the studies in rats were limited to effects in the kidneys, namely, with renal tubular degeneration/regeneration with deposition of crystals in the collecting tubules and ducts at 300 and ≥100 mg/kg/day in the 13-and 26-week studies, respectively. At the NOAEL for the 13- and 26- week studies, systemic exposures (AUC) to BH_4 in males/females were approximately 6-/7- and 2-/2-fold higher than that expected in humans.

No effects considered to be adverse were identified in the studies conducted in marmoset monkeys, in which the maximum tested doses in the 2- 13 and 39-week studies corresponded to systemic exposures (AUC) to BH_4 in males/females of approximately 6-/10-, 2-/3- and 8-/5- fold, respectively, higher than that expected in humans. However, during the 39-week study, there were 12 early deaths (unscheduled euthanasia and animals found dead). Animals who died before D30 were replaced to maintain a sufficient number of animals in each group in the study.

None of the unscheduled deaths observed in the 39-week studies in monkeys were considered related to administration of sepiapterin by the Applicant due to the absence of consistent gross and/or microscopic changes and lack of a pattern. It is true that these early deaths had no consistent gross or microscopic changes but 4 deaths also occurred in the vehicle control group which could have biased the interpretation of deaths occurring in treated groups. 3 females died in the low dose group (D178, 273, 233), 3 animals died in the mid-

dose group (1 male at D27, 2 females at D264 and D274) and 2 females in the high dose group (at D103 and D32), several causes of death were not determined. Some deaths were also noted in shorter studies in marmoset studies. In 14-day study, one female at 100 mg/kg/day (low dose group) died at D7. In the 13-week, 3 deaths occurred: one female in control group at D19, 2 deaths at 100 mg/kg/day (1 male at D30 and 1 female at D85) and no death at the highest dose of 300 mg/kg/day. No microscopic findings were observed in the 39-week study. However, a significant number of animals died during the study (12 animals: 4 females in the control groups and 8 animals in treated groups).

In response to other concerns on the 12 deaths observed in the 39-week study, the Applicant provided clarifications on the respective causes of death, which were considered to be the following:

Table 8. Unscheduled Deaths in the 9-Month Repeat-Dose Toxicity Study in Marmoset Monkeys

Group (Dose Level)	Sex	Animal ID	Fate	Cause of Moribundity/Death
1 (Vehicle Control)	M	A2357	Moribund sacrifice on Study Day 62	Leg fracture
	F	A4181	Found dead on Study Day 51	MWS
		A4193	Found dead on Study Day 29	MWS
		A8269	Moribund sacrifice on Study Day 251	MWS
2 (30 mg/kg/day)	M	B8233	Moribund sacrifice on Study Day 178	Sepsis
	F	B4911	Found dead on Study Day 273	Blood collection
		B2374	Moribund sacrifice on Study Day 233	Pneumonia
3 (100 mg/kg/day)	M	C8244	Found dead on Study Day 27	Sepsis
	F	C2339	Moribund sacrifice on Study Day 264	Ulceration of colon
		C8259	Found dead on Study Day 274	Blood collection
4 (300 mg/kg/day)	F	D2331	Found dead on Study Day 103	MWS
		D4199	Found dead on Study Day 32	MWS

MWS = Marmoset Wasting Syndrome

As justified by the Applicant, historical control data could not be provided as this had not been provided by the contract research organisation due to the limited number of marmoset studies conducted by this laboratory. Based on literature data, Marmoset Wasting Syndrome is one of the most common diseases in marmoset with 50% to 80% incidence in captive colonies.

When deaths were attributed to Marmoset Wasting Syndrome, this was based on in-life observation of adverse clinical signs and/or body weight loss. Evidence for MWS was based on published literature.

The Applicant therefore considered that the deaths were not sepiapterin-related and that there is no need to amend the SmPC Section 5.3 and the RMP Part II: Module SII in this respect. This has been agreed.

In terms of genotoxicity and carcinogenicity, sepiapterin was not genotoxic based on negative results in the bacterial reverse mutation (Ames) assay and sepiapterin did not induce chromosome aberrations in cultured human lymphocytes in the presence of rat liver S9. Notwithstanding, without metabolic activation, sepiapterin induced structural chromosomal aberrations following 20-hour continuous treatment while the conclusion remained equivocal following the short (3-hour) treatment period. Because of this *in vitro* signal was observed in the chromosomal aberration assay, two *in vivo* mammalian toxicity evaluations (micronucleus assay and comet assay) were conducted in male Sprague Dawley rats administered sepiapterin as advised in ICH S2(R1).

In the micronucleus assay, sepiapterin was dosed at 100, 300, 1000 and 2000 mg/kg/day for 3 days, and no increase in the frequency of micronucleated polychromatic erythrocytes (MN-PCE) was observed for male rats administered sepiapterin at any dose level, and there was no evidence of a dose response. The lack of an effect on polychromatic erythrocyte (PCE) frequency indicates there was no bone marrow cytotoxicity.

However, in Comet assay associated to micronucleus assay, a small but statistically positive increase in DNA damage in liver was observed in rats administered 1000 mg/kg/day sepiapterin compared to the vehicle control group. Exposure of BH_4 at 2000 mg/kg/day is lower than at 1000 mg/kg/day. Therefore, exposure at 1000 mg/kg is therefore considered the highest exposure in the study. However, this finding could be considered not biologically relevant since statistically significant DNA damage values at 1000 mg/kg/day were below the mean ±standard error of the mean (±SEM) of the Testing Facility historical control data.

Additionally, sepiapterin is not expected to be carcinogenic in humans because *in vivo*, sepiapterin is rapidly metabolized to BH_4. The WOE described to characterize the carcinogenicity potential presented by the Applicant was considered acceptable. In January 2021, as part of Scientific Advice (EMEA/H/SA/4715/1/2020/III), the CHMP previously agreed that further experiments would be of no value in assessing the long-term oncogenic potential in man. In 26-week rat study, several hyperplasias were noticed. The Applicant discussed hyperplasia observed in kidney and concluded this hyperplasia of the papilla in kidney is not considered as pre-neoplastic change. The discussion presented could be considered acceptable. The Applicant discussed also hyperplasia observed in pituitary glands and concluded that this hyperplasia is most likely incidental and spontaneous in this age of laboratory SD rats and unlikely related to treatment with sepiapterin, the rational is also acceptable. Other hyperplasias were observed but were only present in one treated animal or in one control and one treated animal. No increased incidence of hyperplasias were noted in treated group compared in control group. Findings of hyperplasia observed in long-term studies was not observed in animal studies with sapropterin and therefore was not studied in carcinogenicity studies performed with sapropterin.

In addition, results of a 26-week carcinogenicity study in transgenic mice were submitted. *S*epiapterin was administrated for 26 weeks in Tg mice at dose of 30, 100 and 300 mg/kg/day in males and 100, 300 and 1000 mg/kg/day in females*.* No carcinogenic effect related to sepiapterin administration was observed at any dose level in males or females in this study. This result reinforces the WOE approach submitted by the Applicant which suggests that the carcinogenicity risk with sepiapterin is low.

Reproductive toxicity comprised studies on fertility and early embryonic development (rat), embryo-foetal development (rat and rabbit), pre- post-natal development (rat) and juvenile animal studies (rat).

According to the Applicant, the studies revealed no toxicities other than non-adverse reductions in maternal body weight and food consumption in rabbits.

Regarding the juvenile animal studies, it is noted that no clear pharmacokinetic or toxicological rationale was given to justify the initiation of treatment in such immature animals, considering, e.g., that a human term neonate is equivalent to a PND10 rat based on an overall interspecies comparison of CNS and reproductive organs. It is also noted that the kidney toxicity reported in the 13-week study in adult rats at 300 mg/kg/day

could have led to irrelevant toxicity based on the timing of kidney maturation in both species. However, the final results did not highlight any treatment-related effect at doses up to 30/300 mg/kg/day on investigated parameters including growth, neurobehavior (motor activity, acoustic startle habituation, and learning and memory in Morris water maze), oestrous cycles and sperm parameters (motility, concentration, and morphology). Whereas sexual maturation was not affected in females, preputial separation was significantly delayed in males but this finding was reported to lie within historical control range. In the juvenile toxicity study, dose levels were adjusted on PND 29 to account for an age-related variation in exposure levels to BH_4 in animals aged PND 4 and PND 9, due in part to higher pre-dose levels of BH_4. Indeed, the exposure levels measured at 30 and 100 mg/kg in the 6-day PK study were 5 to 15 times higher than those achieved in the 14-day, 13-week and 26-week toxicity studies in adult animals. In the juvenile toxicity study, AUC values on the day of dose escalation (PND29) were 1.3 to 2.3 times higher than those attained after the first dose in the 13-week toxicity study. However, they decreased over time to levels up to 1.1-fold higher than on Day 91 in the 13-week toxicity study. In particular, exposure levels at 300 mg/kg were only half those reached at the same dose level associated with kidney lesions in the 13-week toxicity study. Therefore, it cannot be excluded that the absence of kidney findings in juvenile rats was due to insufficient exposure levels at 300 mg/kg. It is also noted that the kidney was identified as a target organ in toxicity studies conducted in rats with sapropterin, without corresponding warning for humans in SmPC (see EPAR for KUVAN). Based on exposure levels measured on PND 70 at the NOAEL of 30/300 mg/kg/day, the safety margin in juvenile rats ranges from 3.6 to 4.1.

No further juvenile animal studies have been considered needed in accordance with the Paediatric Investigation Plan.

Sepiapterin had no phototoxic potential based on an *in vitro* phototoxicity study.

No immunotoxicity assessments were performed as no sepiapterin-related effects on the immune system were observed in repeat-dose mammalian toxicology studies.

A WOE assessment including chemistry (structure activity assessment, extraction, conversion, sublimation, and solubility), nonclinical safety data (toxicology and safety pharmacology), PK data, clinical data, pharmacologic activity, and literature data including assessment of metabolite BH_4, concluded that sepiapterin's potential risk for abuse is very low.

Process-related impurities were observed in sepiapterin DS and were satisfactorily qualified in toxicology studies. In addition, potential degradants in sepiapterin DP were also qualified in toxicology studies.

Based on the 28-day rat toxicology studies, the qualified levels of all of the impurities are at or above the specifications proposed in Module 3. The safety and quality data indicate there is no apparent mutagenic risk for humans associated with sepiapterin.

Sepiapterin $PEC_{surfacewater}$ value is above the action limit of 0.01 μg/L. Sepiapterin is a naturally occurring, endogenous substance; expected to be present in the environment. Sepiapterin is not a PBT substance as log K_{ow} does not exceed 4.5.

Therefore, Sephience oral powder 250 mg and 1000 mg, is not expected to pose a risk to the environment.

Precautionary and safety measures taken to reduce any risk to the environment by including the general statement on the SmPC and PL were applied, according to the Guideline on the environmental risk assessment of medicinal products for human use EMEA/CHMP/SWP/4447/00, 2006.

2.5.7. Conclusion on the non-clinical aspects

Overall, the toxicological profile of sepiapterin is well characterized. Considering all available information, the toxicological studies demonstrate a safety profile that may be considered supportive of the clinical use of the highest proposed dose of 60 mg/kg/day sepiapterin for the treatment of HPA in adult and paediatric patients with PKU. From a non-clinical point of view, the application is approvable.

2.6. Clinical aspects

2.6.1. Introduction

GCP aspects

The Clinical trials were performed in accordance with GCP as claimed by the Applicant.

The Applicant has provided a statement to the effect that clinical trials conducted outside the Community were carried out in accordance with the ethical standards of Directive 2001/20/EC.

Table 9. Tabular overview of clinical studies

Population	Study No. Location Publication	Study Phase/Type	Dose Regimen	N
PKU	PKU-002 Australia, Georgia (Bratkovic 2022)	Phase 2: randomized, double crossover, open-label, active-controlled (≥18 years of age)	7 days of sepiapterin 60 mg/kg, sepiapterin 20 mg/kg, sapropterin 20 mg/kg/day	24
	PTC923-MD-003-PKU Global	Phase 3: multicenter, 2-part, double-blind, pbo-controlled, randomized Part 1: 14-day open-label sepiapterin assessment of responsiveness (all ages) Part 2: randomization (1:1) to either 6 weeks sepiapterin or pbo (≥2 years of age)	Part 1: sepiapterin 7.5 mg/kg/day for subjects 0 to <6 months of age, 15 mg/kg for subjects 6 to <12 months of age, 30 mg/kg/day for subjects 12 months to <2 years of age, 60 mg/kg/day for subjects ≥2 years of age Part 2: sepiapterin: 20 mg/kg/day for Weeks 1-2, 40 mg/kg/day for Weeks 3-4, 60 mg/kg/day for Weeks 5-6 or pbo	157
	PTC923-MD-004-PKU[a] Global	Phase 3: open-label, long-term extension, safety, efficacy, QOL (all ages)	Sepiapterin 7.5 mg/kg/day for subjects 0 to <6 months of age, 15 mg/kg/day for subjects 6 to <12 months of age, 30 mg/kg/day for subjects 12 months to <2 years of age, 60 mg/kg/day for subjects ≥2 years of age	104[a]
	PTC923-401 Global	Phase 3b, open-label, neurocognitive outcomes, long-term (<12 years of age)	Sepiapterin 7.5 mg/kg/day for subjects 0 to <6 months of age, 15 mg/kg/day for subjects 6 to <12 months of age, 30 mg/kg/day for subjects 12 months to <2 years of age, 60 mg/kg/day for subjects ≥2 years of age	56[b]
Healthy volunteers	PKU-001 Australia (Smith 2019b, Smith 2019a)	Phase 1: single (Part A) and multiple (Part B) dose escalation, PK, PD, food effect	Part A: single doses of sepiapterin 2.5, 7.5, 20, 40, or 80 mg/kg; sapropterin 100 mg tablets; or pbo Single oral doses of sepiapterin 10 mg/kg in fed and fasted state Part B: sepiapterin 5, 20, or 60 mg/kg/day or pbo PO QD ×7 days	83
	PTC923-MD-005-HV Australia (Gao 2023)	Phase 1: relative bioavailability, safety and tolerability, and palatability	Part A: Phase 1/2 formulation: 20 mg/kg and 60 mg/kg Phase 3 formulation: 20 mg/kg and 60 mg/kg Part B: Phase 3 formulation: 20 mg/kg (fasted and high-fat fed), 60 mg/kg (fasted and high-fat fed)	32
	PTC923-MD-007-HV UK	Phase 1: single-dose, PK, tolerability, food effect, ethnobridging (Japanese vs non-Japanese)	Cohort 1, 4: Japanese and non-Japanese: single dose of sepiapterin 20 mg/kg (fed) Cohort 2, 5: non-Japanese: single dose of sepiapterin 40 mg/kg (fed); Japanese: 2 single oral doses of sepiapterin 40 mg/kg (1 under fed conditions, 1 under fasted) Cohort 3: Japanese and non-Japanese: single oral dose of sepiapterin 60 mg/kg (fed)	60
	PTC923-MD-008-HV US	Phase 1: singe-dose AME (≥18 years of age)	Single dose of 4000 mg sepiapterin containing ~100 µCi ^{14}C-sepiapterin	8

	PTC923-DDI-101-HV US	Phase 1: DDI, effect of sepiapterin on BCRP	Period 1: single dose of sepiapterin 20 mg/kg Period 2: single dose of curcumin 2 g+sepiapterin 20 mg/kg Period 3: single dose of rosuvastatin 10 mg Period 4: single dose of rosuvastatin 10 mg+sepiapterin 60 mg/kg	29
PBD	PBD-001 US	Phase 1/2: safety, PK, preliminary efficacy	Cohort 1: sepiapterin 2.5 mg/kg/day PO BID ×7 days, followed by a 3 (±1) day washout period, then sepiapterin 10 mg/kg/day PO BID ×7 days Cohort 2: Sepiapterin 5 mg/kg/day PO BID ×7 days, followed by a 3 (±1)-day washout period, then sepiapterin 20 mg/kg/day PO BID ×7 days	8
GAS	GAS-001 US	Phase 2: efficacy, safety	Sepiapterin 20 mg/kg/day (10 mg/kg BID) or pbo for 14 days	21

Abbreviations: AME, absorption, metabolism, and excretion; BCRP, breast cancer resistance protein; BH_4, tetrahydrobiopterin; DDI, drug-drug interaction;
N, number of subjects; PBD, primary BH_4 deficiency; pbo, placebo; PD, pharmacodynamic; PK, pharmacokinetic; PKU, phenylketonuria; QOL, quality of life;
UK, United Kingdom; US, United States
a Study PTC923-MD-004-PKU is ongoing. As of the 22 September 2024 data cutoff date, there are 169 subjects enrolled.
b Target subject enrolment. Study is ongoing at the time of submission.

2.6.2. Clinical pharmacology

2.6.2.1. Pharmacokinetics

Absorption

Following oral administration, sepiapterin is quickly absorbed (peak plasma concentrations occur approximately 1 to 3 hours postdose) and is rapidly and extensively converted to BH_4. Plasma sepiapterin concentration is typically less than 1% or 2% of BH_4 and declines below the lower limit of quantitation (LLOQ; 0.75 ng/mL) by 12 hours postdose.

The Phase 1/2 and Phase 3 formulations of sepiapterin were bioequivalent when administered at doses of 20 mg/kg. The 90% CIs of the primary PK parameters for the major circulating active moiety BH_4 were within the no effect limit of 80% to 125% at sepiapterin dose 20 mg/kg, and the lower bounds of the 90% CI were slightly below 80% at dose 60 mg/kg (range 73.49% to 75.52%). No apparent difference in the absorption rate and elimination rate between the 2 formulations was observed. Similar intersubject variability for the primary PK parameters of the 2 formulations was observed. Based on the population PK model, the fraction of absorption/biotransformation was estimated to be 30% less in patients with PKU when compared to healthy subjects.

Clinical data collected for both the Phase 1/2 formulation and the Phase 3 formulation in healthy volunteers demonstrated that administration of sepiapterin with food results in increased sepiapterin and BH_4 exposure. Greater increases in exposure were observed following administration with meals of higher fat content.

Distribution

In vitro binding of sepiapterin and BH_4 to human plasma proteins was relatively low. The geometric % mean of sepiapterin bound to human plasma protein was 15.4% at 0.1, 1, and 10 µM when stabilized with 0.1% dithiothreitol, and the mean % BH_4 plasma protein binding was 41.3%, 33.0%, and 24.1% at 2, 5, and 15 µM, respectively, when stabilized with 0.5% β-mercaptoethanol.

Elimination

Plasma sepiapterin is rapidly metabolized to BH_4, and peak BH_4 concentrations are achieved approximately 4 hours after the oral administration of sepiapterin (under both fed and fasted states).

Sepiapterin is rapidly converted to BH_4 in vivo by a 2-step reduction primarily mediated by sepiapterin reductase (SR)/carbonyl reductase and dihydrofolate reductase (DHFR) in the pterin salvage pathway. The plasma concentration of sepiapterin is negligible following oral administration (C_{max} ~3 to 5 ng/mL in humans after 7-day repeat-dose administration at 60 mg/kg/day) and generally <1% or 2% of BH_4 C_{max}. BH_4 is oxidized during catalytic aromatic amino acid hydroxylation and regenerated by pterin-4α-carbinolamine dehydratase and dihydropteridine reductase.

In an absorption, metabolism, and excretion (AME) study in healthy human volunteers, orally administered [^{14}C]-sepiapterin was extensively metabolized and predominantly excreted in faeces (26.18%), with a smaller fraction of the dose excreted in urine (6.71%). The majority of radioactivity was excreted during the first 48 hours postdose. While the total recovered radioactivity was relatively low, data from combined excreta of faeces and urine suggested that the majority of recoverable radioactivity was already excreted by 240 hours. There was 54.5% radioactivity remaining in the test system after incubating [^{14}C]-sepiapterin at 100 µM in human intestinal microbiota anaerobically for 48 hours, indicating formation of volatile metabolites. The low total mass recovery in the human AME study is likely due to formation of volatile metabolites in human intestine. The renal clearance of total radioactivity derived from [^{14}C]-sepiapterin is 1.536 L/h (25.6 mL/min).

Major metabolic pathways included hydrogenation/reduction, dehydrogenation, oxidative deamination, dehydration, oxidation, methylation, and C-C bond side chain cleavage, alone or in combination.

Dose proportionality and time dependencies

Both BH_4 C_{max} and AUCs ($AUC_{0\text{-}24h}$ and $AUC_{0\text{-}inf}$) increase approximately dose proportionally in the range of 5 to 20 mg/kg and less than proportionally in the dose range 20 to 60 mg/kg. There is no accumulation of BH_4 following repeated dosing of sepiapterin up to 60 mg/kg.

Special populations

Table 10. Special populations

	Age 65-74 (Older subjects number /total number)	**Age 75-84 (Older subjects number /total number)**	**Age 85+ (Older subjects number /total number)**
PK Trials	0	0	0

Lower BH_4 C_{max} and AUCs were observed in patients with PKU when compared with healthy subjects receiving the same dose of sepiapterin 60 mg/kg/day.

There was no clear trend of age dependency of BH_4 exposures (C_{max} and $AUC_{0\text{-}24h}$) with the bodyweight-based dose regimen in patients ≥2 years of age.

The effect of age on apparent central volume of distribution (Vc/F) was retained in the final population PK model of BH_4, although no trends were observed between individual random effects of apparent systemic clearance (CL/F) and age suggesting that the elimination of BH_4 is not age dependent. Age was not retained as a significant covariate for potential maturation on the elimination phase.

The safety and efficacy of sepiapterin in patients 65 years and older has not been established in clinical studies.

No dose adjustment is warranted for Japanese patients with PKU. There was no significant difference in BH_4 exposure, intersubject variability, and dose proportionality for Japanese and non-Japanese subjects.

The covariate analysis conducted as part of the development of the population PK model showed that sex was not a statistically significant predictor of volume or clearance.

Sepiapterin has not been studied in subjects with renal or hepatic impairment. The PK of sepiapterin and BH_4 are not expected to be affected by hepatic or renal insufficiency. Sepiapterin is rapidly absorbed and converted to BH_4 via a 2-step process, primarily mediated by SR (likely assisted by carbonyl reductase) and DHFR. Metabolism is not mediated by CYP enzymes. In addition, the fraction of dose eliminated via renal clearance is very small (6.71%). The effects of renal and hepatic function biomarkers (alanine aminotransferase [ALT], aspartate aminotransferase [AST], alkaline phosphatase [ALP], albumin, bilirubin, blood urea nitrogen [BUN], serum creatinine, and estimated glomerular filtration rate [eGFR]) had no statistically significant impact on BH_4 PK and were not retained as covariates for the population PK model ($p>0.05$) when assessed during the development of the population PK model for BH_4. Dedicated studies to assess the effect of renal impairment (PTC923-RI-103-HV) and hepatic impairment (PTC923-HI-104-HV) on the pharmacokinetics (PK) and safety of sepiapterin and the active metabolite BH4 are ongoing, a Type-II variation will be submitted post-authorisation to include data upon completion.

Sepiapterin has not been studied in pregnant or breastfeeding women. It is not known whether sepiapterin is present in human milk. The Applicant confirms that one follow-up questionnaire (FuQ) including both pregnancy and lactation will be used. The FuQ is designed to collect specific key information relating to both pregnancy and lactation. The FuQ is added as a routine pharmacovigilance activity for the missing information use during pregnancy and lactation to the EU-RMP version 0.3. The implementation of one FuQ covering both pregnancy and lactation is considered acceptable.

Pharmacokinetic interaction studies

The potential of sepiapterin and BH_4 as victims or perpetrators of BCRP-mediated drug interactions was investigated clinically in adult healthy subjects with the following findings:

- Oral coadministration of curcumin, a BCRP inhibitor, and sepiapterin in healthy adults resulted in slight increases in average AUCs and C_{max} of BH_4 by approximately 20% to 24%, after a single dose. This increase is not clinically relevant, and no dose adjustment of sepiapterin is warranted.
- Oral coadministration of sepiapterin and rosuvastatin, a BCRP substrate, had no impact on rosuvastatin exposure, and no dose adjustment is warranted.

Pharmacokinetics using human biomaterials

Drug-drug interaction studies indicate that sepiapterin and the major circulating active moiety BH_4 are unlikely to be either perpetrators or victims of CYP450 enzyme or transporter mediated drug-drug interactions *in vivo*. As such, no dose adjustment would be required if inhibitors or substrates of CYP or transporter enzymes are co-administered with sepiapterin.

Based on *in vitro* data, sepiapterin was not a substrate of efflux transporter multidrug resistance protein 1 (MDR1) or of uptake transporters organic anion transporting polypeptide (OATP)1B1, OATP1B3, multidrug and toxin extrusion (MATE)1, MATE2-K, organic anion transporter (OAT)1, OAT3, organic cation transporter (OCT)2.

Sepiapterin did not inhibit the efflux transporters MDR1 or bile salt export pump or the uptake transporters MATE1, MATE2-K, OAT1, OAT3, OATP1B3, OCT1, and OCT2. Sepiapterin was a weak *in vitro* inhibitor of OATP1B1.

Based on in vitro data, BH_4 was a substrate and an inhibitor of BCRP and MDR1. BH_4 was a substrate of the MATE2-K uptake transporter only at the highest tested concentration and was unlikely to be a substrate of uptake transporters MATE1, OAT1, OATP1B1, OATP1B3, OCT1, and OCT2. BH_4 did not inhibit the efflux transporter BSEP or uptake transporters ENT1, ENT2, MATE1, MATE2-K, OAT1, OAT3, OATP1B1, and OATP1B3. BH_4 weakly inhibited OCT1 and OCT2.

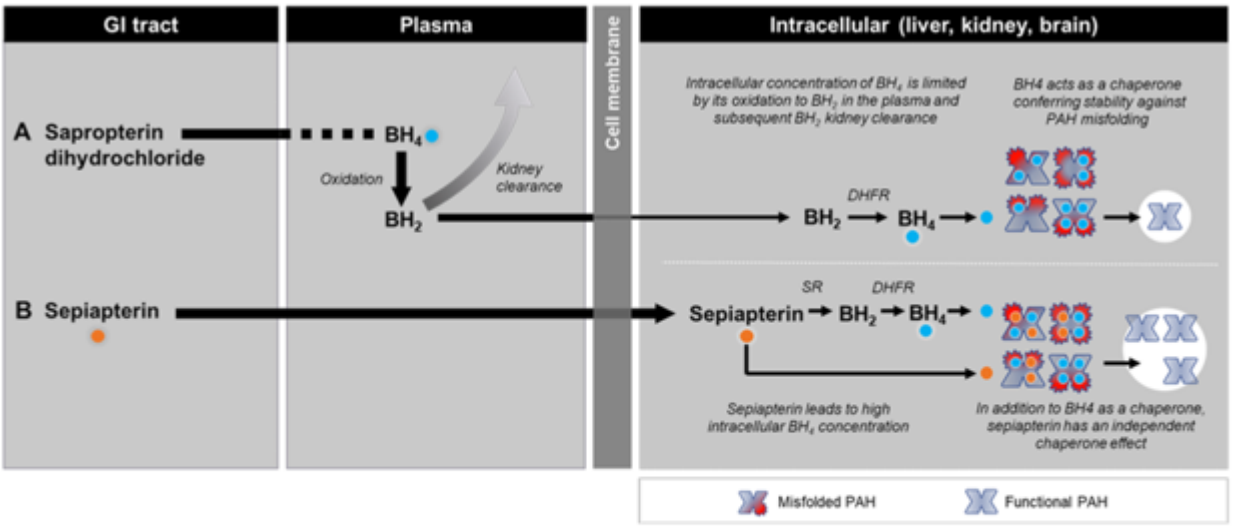
2.6.2.2. Pharmacodynamics

Mechanism of action

Sepiapterin (previously known as PTC923 or CNSA-001) is a new chemical entity, an exogenously synthesized, structurally equivalent version of the biologically produced compound sepiapterin being developed for the treatment of hyperphenylalaninaemia (HPA) in patients with phenylketonuria (PKU). Sepiapterin functions in a dual capacity as (1) an endogenous precursor of naturally occurring tetrahydrobiopterin (BH_4), and (2) a pharmacologically active chaperone for phenylalanine hydroxylase (PAH) native state conformation, conferring stability against thermal unfolding and resulting in enhanced activity and prolongation of PAH function. PAH catalyses the conversion of the essential amino acid phenylalanine (Phe) to tyrosine (Tyr) with the help of the cofactor BH_4.

Therefore, in addition to the rapid conversion of sepiapterin to BH_4, sepiapterin itself exhibits an added chaperone effect on misfolded PAH to improve enzymatic activity and stabilize variant PAH against thermal degradation. Importantly, and in contrast to lipophobic sapropterin, sepiapterin is actively transported intracellularly, elevating intracellular concentrations of the parent drug and its metabolite, BH_4 (Sawabe 2008, Smith 2019b). The greater bioavailability of sepiapterin increases the ability to restore the function of misfolded PAH, thereby augmenting its enzymatic activity and functional capacity to lower blood Phe concentration.

Figure 2: **Mechanism of Action of sapropterin and sepiapterin**



Abbreviations: BH_2, dihydrobiopterin; BH_4, tetrahydrobiopterin; DHFR, dihydrofolate reductase; PAH, phenylalanine hydroxylase; SR, sepiapterin reductase
Note: PKU and associated HPA result from pathologic variants within the *PAH* gene resulting in misfolding of the enzyme and reduced enzyme activity. Sepiapterin actively transported into the cell resulting in pharmacologic levels of intracellular BH_4 and sepiapterin. Sepiapterin and BH_4 increase PAH activity by acting as pharmacologic chaperones to restore conformation structure of the enzyme and consequently promoting conversion of Phe to Tyr.
Source: (Sawabe 2008, Gersting 2010, Smith 2019b)

Primary and Secondary pharmacology

The most relevant data to support the primary PD data was derived from the pivotal Phase 3 study: PTC923-MD-003-PKU.

The primary efficacy endpoint is the mean change in blood Phe levels from baseline (average of Day -1 and Day 1 pre-dose) to Weeks 5 and 6 (average over a 2-week period) in the Part 2 double-blind phase.

The primary analysis was performed in the stratum of participants with a mean percent reduction in blood Phe levels of ≥30% during Part 1. As specified in the SAP, only if the primary analysis was statistically significant, a test of the same endpoint based on the FAS would be performed.

In Part 2, a statistically significant ($p<0.0001$) difference in the mean change in blood Phe levels from baseline to Weeks 5 and 6 was observed following treatment with sepiapterin compared with placebo in the primary analysis population (the stratum of participants in the FAS who demonstrated a ≥30% reduction in blood Phe levels during Part 1) (Table 11).

Table 11. Mean change in blood Phe levels (µmol/L) from baseline to Week 5 and Week 6 in Part 2 (Full Analysis Set with Phe reduction from baseline ⩾30% during Part 1)

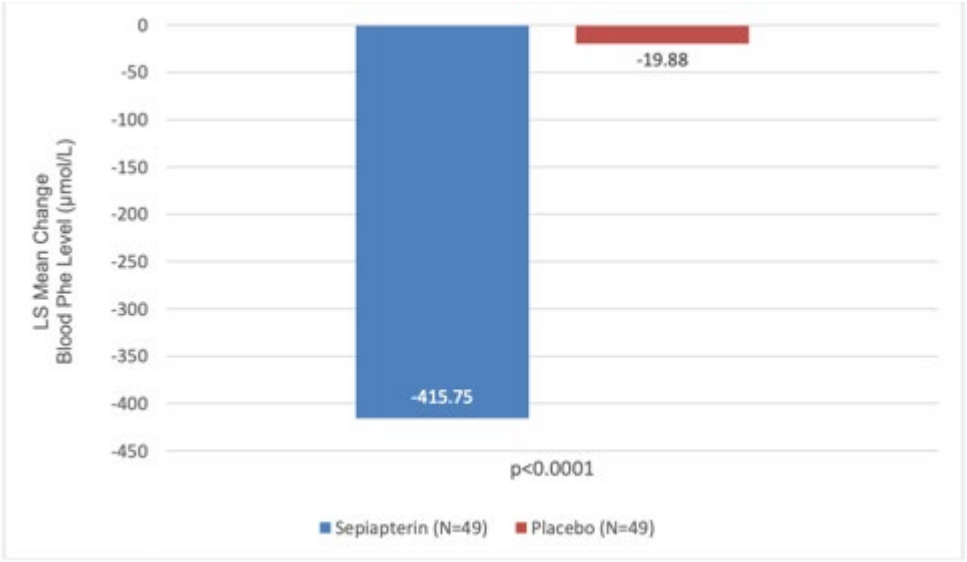
	Sepiapterin (N=49)	Placebo (N=49)	Difference Sepiapterin vs Placebo	P Value
Baseline				
n	49	49		
Mean (SD)	646.11 (253.007)	654.04 (261.542)		
Weeks 5 and 6				
n	49	49		
Mean (SD)	236.04 (174.942)	637.85 (259.886)		
Mean change from baseline (SD)	-410.07 (204.442)	-16.19 (198.642)		
LS mean estimate for the mean change from baseline				
LS mean (SE)	-415.75 (24.066)	-19.88 (24.223)	-395.87 (33.848)	<0.0001
95% CI	(-463.52, -367.97)	(-67.97, 28.21)	(-463.07, -328.66)	

Abbreviations: CI, confidence interval; LS, least squares; MMRM, mixed model for repeated measures; Phe, phenylalanine; SD, standard deviation, SE, standard error
Note: Baseline is the average of Day -1 and Day 1 blood Phe levels in Part 2.
LS means, SEs, CIs, and p values are based on the MMRM model on change from baseline in blood Phe with treatment, baseline Phe stratum (<600 or ≥600 µmol/L), visit, and treatment-by-visit interaction; baseline blood Phe as fixed effects; and a random participant effect with an unstructured covariance matrix.

Baseline mean Phe levels were similar between the sepiapterin and placebo arms. By Week 6, mean blood Phe levels decreased significantly in the sepiapterin arm (LS mean change of -415.75 µmol/L, a 63% reduction vs baseline), whereas Phe levels remained relatively unchanged in the placebo arm (LS mean change of -19.88 µmol/L, a 0.8% increase vs baseline).

In the stratum of participants in the FAS who demonstrated a ≥30% reduction in blood Phe levels during Part 1, 89.8% (44/49) of the participants in the sepiapterin group achieved a reduction of ≥30% during Part 2 compared to 10.2% (5/49) in the placebo group.

Figure 4: **LS mean change in blood Phe levels from baseline to Week 5 and Week 6 in Part 2 (Full Analysis Set with Phe reduction from baseline ⩾30% during Part 1)**



Abbreviations: Phe, phenylalanine; LS, least squares

Consistent with the results of the primary analysis in the 30% responder population, in the Full Analysis Set (FAS), a statistically significant ($p<0.0001$) difference in the mean change in blood Phe levels from baseline to Weeks 5 and 6 was observed in the sepiapterin arm following treatment with sepiapterin compared with placebo.

Baseline mean Phe levels were similar between the sepiapterin and placebo arms. By Week 6, mean blood Phe levels decreased significantly in the sepiapterin arm (LS mean change of -289.59 µmol/L), whereas Phe levels remained relatively unchanged in the placebo arm (LS mean change of 65.31 µmol/L) (Table 12, Figure 6).

Table 12. Mean change in blood Phe levels (µmol/L) from baseline to Week 5 and Week 6 in Part 2 (Full Analysis Set)

	Sepiapterin (N=56)	Placebo (N=54)	Difference Sepiapterin vs Placebo	P Value
Baseline				
N	56	54		
Mean (SD)	645.59 (246.085)	667.81 (264.574)		
Weeks 5 and 6				
N	56	54		
Mean (SD)	280.74 (236.964)	641.54 (270.157)		
Mean change from baseline (SD)	-364.84 (244.997)	-26.27 (199.384)		
LS mean estimate for the mean change from baseline				
LS mean (SE)	-289.59 (31.528)	65.31 (32.958)	-354.90 (36.435)	<0.0001
95% CI	(-352.00, -227.18)	(0.07, 130.54)	(-427.14, -282.66)	

Abbreviations: CI, confidence interval; LS, least squares; MMRM, mixed model for repeated measures; Phe, phenylalanine; SD, standard deviation; SE, standard error

Note: Baseline is the average of Day -1 and Day 1 blood Phe levels in Part 2.

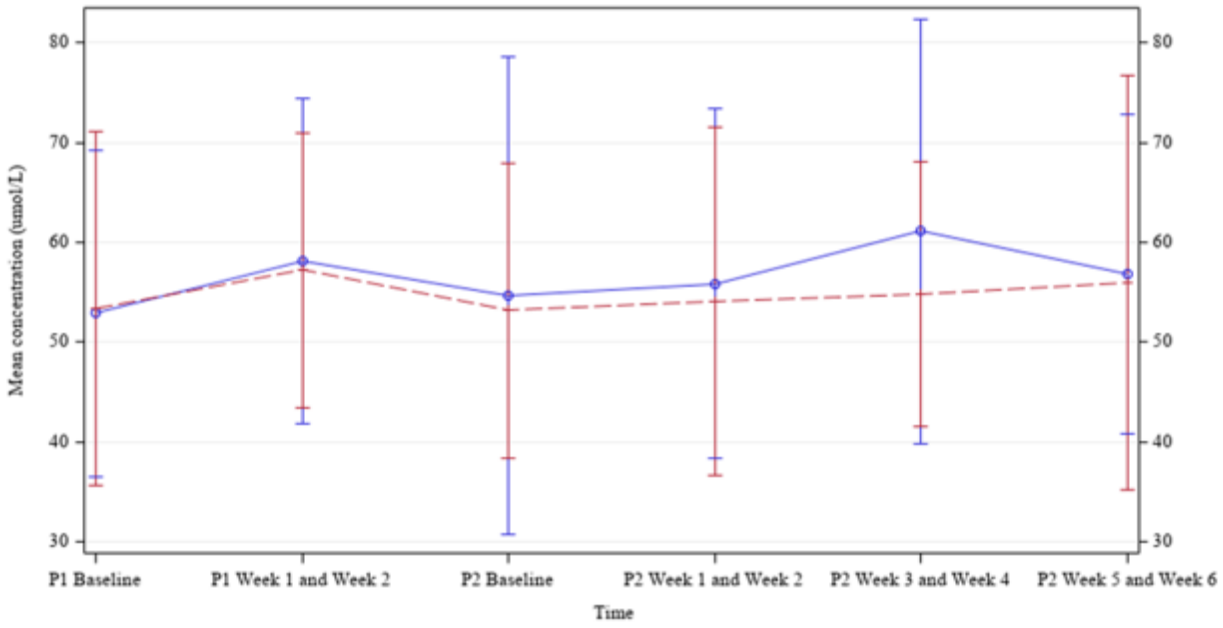
LS means, SEs, CIs, and p values are based on the MMRM model on change from baseline in blood Phe with treatment, baseline Phe stratum (<600 or ≥600 µmol/L), Phe reduction (≥15% to <30% or ≥30%), visit, and treatment- by-visit interaction; baseline blood Phe as fixed effects; and a random participant effect with an unstructured covariance matrix.

In the FAS, 89.8% (44/49) of the participants in the sepiapterin group achieved a reduction of ≥30% during Part 2 compared to 10.2% (5/49) in the placebo group.

Neurological biomarkers in the CSF, namely sepiapterin, BH_4, BH_2, Homovanillic acid (HVA), and 5-hydroxyindoleactic acid (5-HIAA), were assessed in healthy volunteers receiving repeated dose of sepiapterin at 60 mg/kg/day for 7 days in Study PKU-001. Sepiapterin was not detectable in the CSF on Day 1 or Day 7 (PKU-001 CSR). Notable changes were observed for BH_4 and BH_2 in CSF. Administration of sepiapterin 60 mg/kg/day for 7 days resulted in increases in BH_4 and BH_2 from baseline of 1.94-fold and 3.68-fold, respectively. Minimal effects were observed on mean levels (pre-treatment vs posttreatment) of biomarkers of serotonin metabolite 5-HIAA and dopamine metabolite HVA.

In the tyrosine pathway, BH_4 is an essential cofactor in the conversion of Phe to Tyr by PAH. Given the efficacy of sepiapterin in reducing blood Phe concentration via an increase in BH_4 concentration and a chaperone effect to restore PAH activity, change in blood Tyr concentration and Phe:Tyr ratio were used as exploratory endpoints of sepiapterin efficacy in Study PTC923-MD-003-PKU. During Parts 1 and 2, minimal change in Tyr concentration was observed (Figure 5).

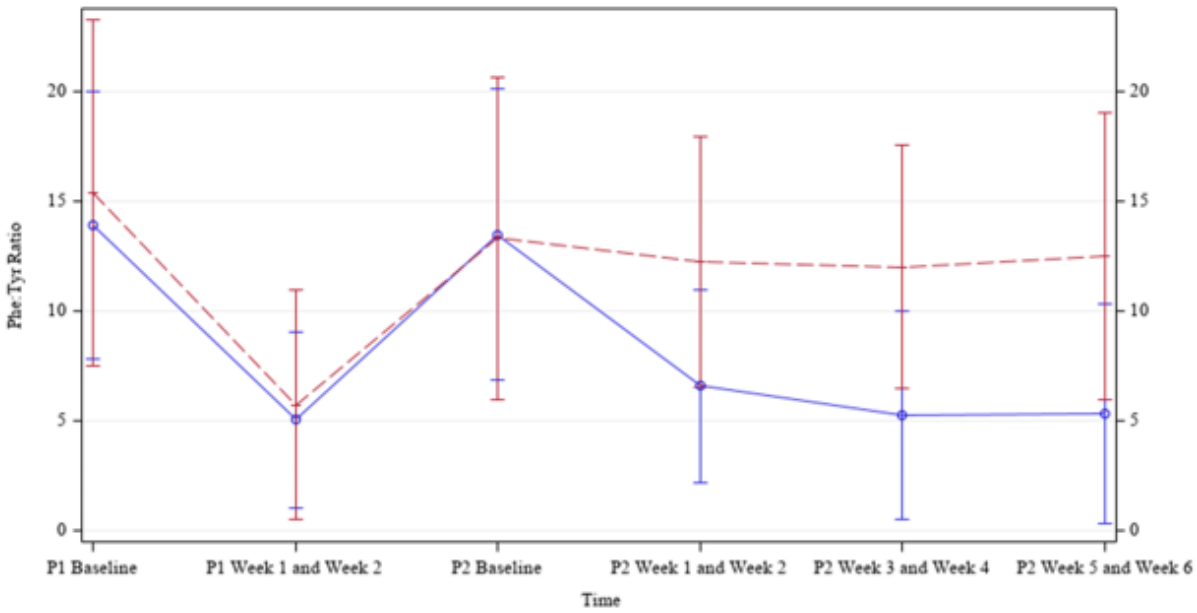
Figure 5: **Mean (SD) Tyr concentration (µmol/L) over time by treatment group (Full Analysis Set)**



Abbreviations: P, part; SD, standard deviation; Tyr, tyrosine
Note: Blue line represents sepiapterin in Part 2; red line represents placebo in Part 2.
Full Analysis Set includes all randomized subjects who were administered at least 1 dose of double-blind study drug in Part 2.

During the study, the Phe:Tyr ratio decreased when sepiapterin was administered (Figure 6). The Phe:Tyr ratio remained relatively unchanged with placebo treatment. In the Primary Analysis Set, the mean change from baseline in the Phe:Tyr ratio by Week 6 was -9.09 and -1.05 with sepiapterin and placebo, respectively (PTC923-MD-003-PKU CSR). In the Full Analysis Set, the mean change from baseline in the Phe:Tyr ratio by Week 6 was -8.16 and -0.80 with sepiapterin and placebo, respectively.

Figure 6: **Mean (SD) Phe:Tyr ratio over time by treatment group (Full Analysis Set)**



Abbreviations: P, part; Phe, phenylalanine; SD, standard deviation; Tyr, tyrosine
Note: Blue line represents sepiapterin in Part 2; red line represents placebo in Part 2.
Full Analysis Set includes all randomized subjects who were administered at least 1 dose of double-blind study drug in Part 2.

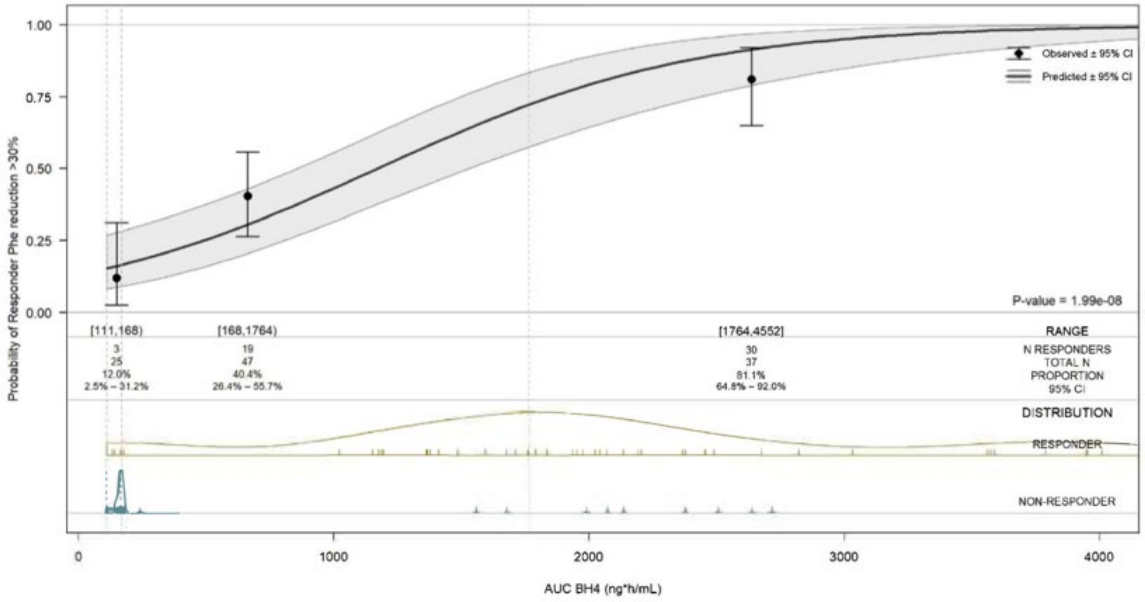
Exposure-response analyses were performed on different subsets of the data collected in Study PTC923-MD-003-PKU to assess the relationship between exposure of BH_4 and the levels of Phe in patients with PKU at 20 mg/kg (Weeks 1 and 2), 40 mg/kg (Weeks 3 and 4), and 60 mg/kg (Weeks 5 and 6) (Report PTC923-2021-022). BH_4 exposures were simulated, and the descriptive statistics of characteristics of the population were included in the exposure-response analysis.

For all efficacy endpoints (≥15% Phe Reduction, ≥30% Phe reduction, Phe concentration <360 µmol/L and Phe concentration <600 µmol/L), BH_4 AUC was a good predictor of Phe reduction ($p<0.001$). All logistic regression models included Part 1 responsiveness as a statistically significant covariate, with an increase of responder probability for patients with ≥30% Phe reduction in Part 1. The odd ratios to reach the efficacy endpoints increased in the range from 8.71 to 151 compared to subjects with Part 1 Phe reduction response between ≥15% and <30%. Additionally, the impact of age, weight, sex, ethnicity, race, previous BH_4 supplements therapy, response to BH_4 supplement treatment challenges, and classic PKU patients have been investigated and were found to have no significant impact on all efficacy endpoints evaluated. Thus, none were retained in the final model. Model predictions for patients with Part 1 responsiveness of Phe reduction ≥30% included the following:

- Probabilities to reach Phe reduction ≥30% in Part 2 increased from 20 mg/kg to 40 mg/kg with probability from 85.4% to 91.9% at their respective means of BH_4 AUC (1445 and 1922 h•ng/mL, respectively). Similar probabilities are predicted at doses of 40 mg/kg and 60 mg/kg (Figure 7).
- Probabilities to reach Phe value <360 µmol/L in Part 2 increased from 20 mg/kg to 40 mg/kg with probability from 75.3% to 84.9% at their respective means of BH_4 AUC (1420 and 1888 h•ng/mL, respectively). Similar probabilities are predicted at doses of 20 mg/kg and 60 mg/kg (Figure 8).

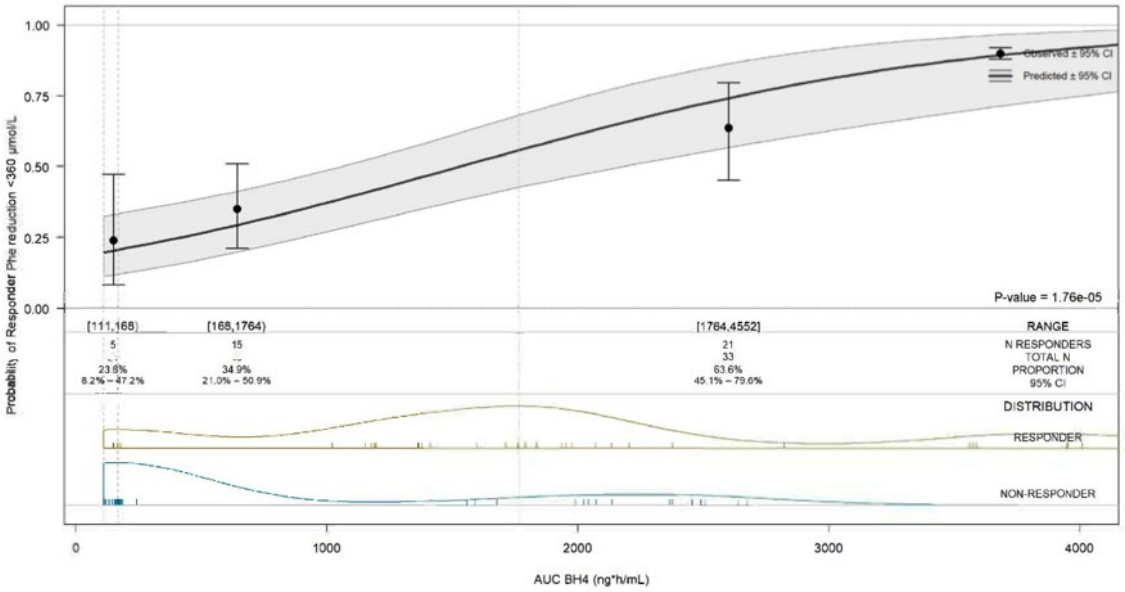
- Probabilities to reach Phe reduction ≥15% and Phe concentration <600 µmol/L in Part 2 were high at a dose of 20 mg/kg, at 94.2% and 96.1%, respectively. The difference in efficacy could not be differentiated with higher sepiapterin dose at 40 or 60 mg/kg (Figure 9).

Figure 7: **Probability of ⩾30% Phe reduction vs AUC of BH_4 in Study PTC923-MD-003-PKU Part 2 at Weeks 5 and 6**



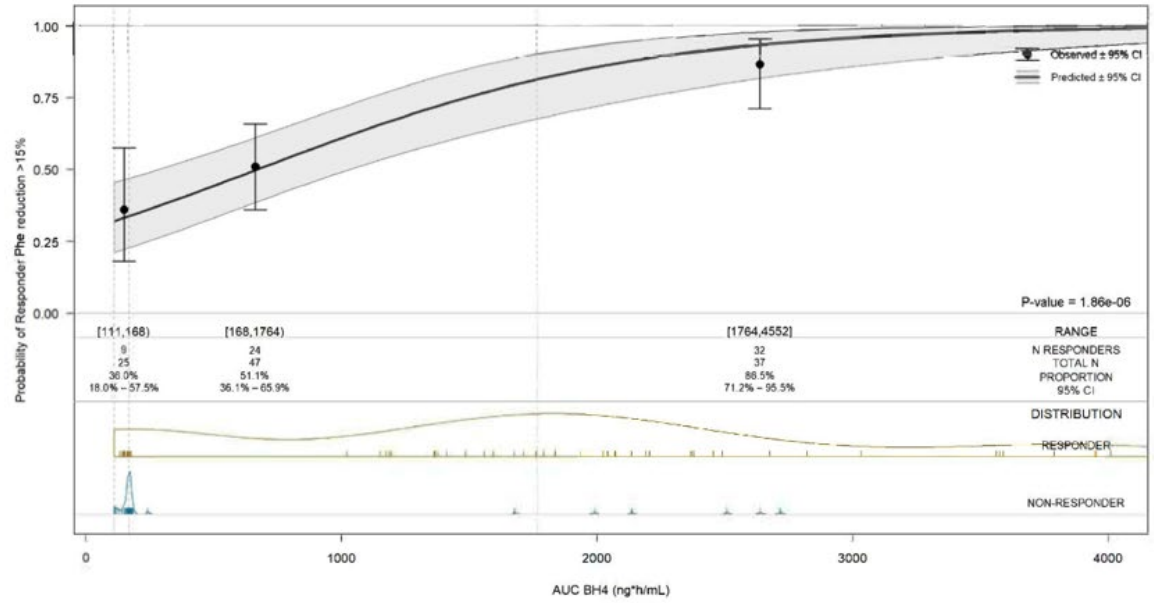
BH4= 5,6,7,8-tetrahydrobiopterin; CI= confidence interval; C_{min} = minimum concentration; N= number of subjects; Phe= phenylalanine

Figure 8: **Probability of <360µmol/L Phe vs AUC of BH_4 in Study PTC923-MD-003-PKU Part 2 at Weeks 5 and 6**



AUC= area under concentration-time curve from time 0 to 24h after dose; BH_4= 5,6,7,8-tetrahydrobiopterin; CI= confidence interval; N= number of subjects; Phe= phenylalanine

Figure 9: **Probability of ≥15% Phe reduction vs AUC of BH_4 in Study PTC923-MD-003-PKU Part 2 at Weeks 5 and 6**



AUC= area under concentration-time curve from time 0 to 24h after dose; BH_4= 5,6,7,8-tetrahydrobiopterin; CI= confidence interval; N= number of subjects; Phe= phenylalanine

Overall, no significant differences were observed between the probabilities of responders at 40 mg/kg and 60 mg/kg. The Part 1 14-day sepiapterin responsiveness test from 60 mg/kg/day treatment of sepiapterin was a

significant covariate for all explored clinical efficacy endpoints (30% Phe reduction, 15% Phe reduction, and probability to reduce Phe level to <600 µmol/L and <360 µmol/L). The strong correlation between the responsiveness test and final clinical treatment effect suggests that the 14-day responsiveness test is a reliable tool to indicate potential clinical benefit for treating patients with PKU.

2.6.3. Discussion on clinical pharmacology

Bioanalytical methods

The Applicant developed two analytical methods for the determination of sepiapterin and BH_4 in human plasma, CSF and urine. Validations were appropriate, with stability well documented and ISR well presented. For plasma, an initial method in human plasma (stabilized with 1% ascorbic acid, 0.1% DTE and 0.01% DETAPAC) was used in the initial studies PKU-01 and PBD-001. Later, an additional method with Acidified Human Plasma (blood samples were treated with 10% ascorbic acid), was also developed. The in-run use of the developed methods was well performed, with the sample analysis being performed within the validated long-term stability conditions. Also, the ISR analysis performed in several of the latter studies always confirmed the quality of the methods, with more than 80% samples complying with the acceptance criteria.

Additional methods were also developed for the DDI study (Rosuvastatin), the mass balance study as well as several PD biomarkers (Phe, Tyr and serotonin). Their validation and use were also acceptable.

Pharmacokinetic data analysis

Classical NCA analysis and population PK modelling were performed and well documented, see below for additional comments on Population PK modelling. Both non-compartmental and PopPK models were used in the several studies. Standard statistical methods were also used in the different comparisons. Overall, the PK analysis is adequate.

Evaluation and qualification of models

Two popPK models and a popPK/PD model were developed during the drug development process. An initial prospective PK model was developed with data from the PKU-001 study and validated with data from a phase 3 study. This model was used for simulating the doses in children to be used in the phase 3 studies. A second PK model was developed using all the available data by September 2023. Overall, the model development procedures seemed adequate, and the final models seem to have good performance, as judged by the presented GOF and VPCs plots. However, several parameters were not logical and co-variables included seem not statistically relevant. Since the Applicant had additional data from the completed Study PTC923-TQT-102-HV and the ongoing Study PTC923-MD-004-PKU (with PK data cut-off date 30 June 2024), a new popPK analysis was developed. Compared to the previously presented model, this one is simpler (with only one compartment) and with different covariables included. For example, the effect of age on the apparent central volume of distribution (Vc/F) is no longer included but a gender effect is now included in the Absorption duration (proposing a 15%, and not clinically relevant, increase in the absorption duration). All covariables are now statistically significant, with the 95%CI not including zero. Also, the model parameter values are now more in line with the ones observed in the initial prospective popPK model. The PK-PD model adds a sigmoid with Imax function. This model seems to underpredict the upper part (upper 95% CI) of the model, as seen in the clinical report, Phe levels vs BH_4 AUC.

When evaluating PK data from sparse and rich sampling from Study PTC923-MD-003-PKU and Study PTC923-MD-004-PKU by age group, it seemed clear that lower BH_4 exposures were obtained in participants <2 years of age. As such, it seemed that the introduction of the maturation function in the prospective popPK model was no longer supported by the available data. Also, the updated popPK model did not require the consideration of the renal and transporter maturation functions utilised to project dose in the prospective model. Although the Applicant argued that the comparable sepiapterin efficacy between participants with PKU <2 years and ≥2 years of age was observed, the conclusions from the updated popPK-PD model was that, at lower doses (20 and 40 mg/kg QD for patients of age ≥2 years, and 7.5, 15, and 30 mg/kg for patients of age < 2 years), comparable but lower percentages of PKU patients could attain the same efficacy targets (greater than 30% Phe reduction, Phe normalization, Phe control, and Phe less than 600 µM). However, more patients would benefit from the treatment across all ages, from neonates to adults, if treated at 60 mg/kg QD. Since the previous round, the Applicant updated the number of patients < 2years of age from study PTC923-MD-004-PKU to 33 (previously 15), 8 aged <1 year, and elaborated on the new data on efficacy provided that no new PK is yet available, thus considering this sufficient to support the dose and efficacy/safety of the treatment in this population without the need to require popPK modelling and extrapolation. In this line of reasoning, the Applicant did not elaborate on the request to further assess possible benefit of a higher dose for children <2 years old based on the popPK model results. As the dose currently used demonstrates consistent decreases in blood Phe levels, maintenance of blood Phe levels below standard goal and tolerance of Phe dietary increases, in line with the results in older patients, this is acceptable.

Of note, no part in the population PK nor the PK-PD model confirms the double mode of action of sepiapterin claimed by the Applicant.

Absorption

The Applicant did not perform an absolute bioavailability study. However, a mass balance study was done showing that 6.7% of the dose is excreted in the urine. This should establish the lower limit of the oral absorption because, since sepiapterin and BH_4 are endogenous substances, they may be further involved in biosynthesis of other biological molecules and not able to be collected in the mass balance study. From the SD/MD ascending dose study, plasma sepiapterin concentrations generally increased with increasing single doses of sepiapterin, with C_{max} achieved in about 1 to 2 hours, showing a fast absorption. However, the conversion from sepiapterin to BH_4 was also rapid and extensive and the Sepiapterin C_{max} was generally less than 2% of BH_4 C_{max}. Plasma BH_4 also increased with increasing single doses of sepiapterin, reaching a peak at about 4 hours. A similar T_{max} was observed when BH_4 is administered in its synthetic form (sapropterin dihydrochloride), indicating a similar overall rate, but with a lower bioavailability.

Although the oral bioavailability is not known, the *in vitro* permeability of sepiapterin in MDCKII cells was low. Sepiapterin is not a substrate of P-GP, thus this seems to be due to its low lipophilicity and difficulty in crossing cell membranes. It may be considered a slightly soluble substance but due to its high doses, and according to the BCS criteria, it is a low solubility drug. So, overall, it may be considered a BCS class IV drug. In addition, sepiapterin is highly metabolized, being almost not observed in the plasma.

ADME study PTC923-MD-008-HV showed 32.9% recovery, which is very low, and only 6.7% elimination in urines, confirming low absorption. Studies in Monkeys and with microbiota (see NC) suggest a strong degradation of sepiapterin in intestine.

Two formulations were used throughout the drug development process: a Phase 1/2 formulation and a Phase 3 formulation. The Phase 3 formulation is also the proposed commercial formulation. In order to bridge studies

between them, a bioequivalence (BE) study was performed between the two formulations at two different doses (20 mg/kg and 60 mg/kg) and after a standard low-fat breakfast. BE was not shown with the parent compound in any of the studied conditions. This may well be due to very high within-subject variability (>30%) and low number of studied subjects ($14 < n < 17$). Regarding the active metabolite, BH_4, BE was demonstrated for the lower dose but not for the higher dose. This may indicate some formulated related differences in the formulation performance, in particular because there is less than proportional linearity and this may be due to solubility issues. However, this seems to be not clinically relevant, and the formulation covariate was also not considered relevant in the popPK model. Overall, since the commercial formulation was used in the Phase 3, this lack of proof of BE at the higher dose is not considered problematic.

Exposition was compared between the main formulations, and BE (with sepiapterin measures) shown at 20 mg/kg, the limits being slightly overreached for BH_4.

Administration of sepiapterin with food increased exposure to sepiapterin and its metabolite BH_4. Greater increases in exposure for both sepiapterin and BH_4 were noted with higher fat content when compared to a low-fat meal. In general, food delayed the absorption of sepiapterin increasing its T_{max} and also the BH_4 T_{max}, especially at the low dose of 20 mg/kg. The effect on BH_4 T_{max} was less evident at 60 mg/kg. In the phase 3 clinical trials, administration of the drug was made with food and the same is proposed in the SmPC.

Distribution

Binding to human plasma proteins was low and independent of the studied concentration for both sepiapterin and BH_4, although the studied concentrations for sepiapterin where higher than the ones observed *in vivo*.

BH_4 Vz/F increased with dose from the starting dose of 2.5 mg/kg, which indicated that the absorption was likely reduced with dose and nonlinear PK were expected. When comparing fed and fasted states at the same dose level, Vz/F reduced when sepiapterin was dosed with food; this is consistent with the observation that food increased BH_4 C_{max} and area under the time-concentration curve (AUC), which suggested oral bioavailability is increased with food. BH_4 Vz/F ranged from 30.3 to 397 L/kg. Compatible with a low extent of distribution, the CSF concentrations of sepiapterin and BH_4 are either neglected or significantly lower than the ones observed in plasma.

Elimination

A mass balance study was performed with a 4000 mg dose of sepiapterin, similar to the one proposed for a 70 kg patient. This is acceptable for a drug showing non-linearity in their PK. Since there is no accumulation in MD, a study in SD is also acceptable. Overall, only 33% of the dosed radioactivity was recovered. Although very low, this is probably related to a formation of volatile metabolites in human intestine, as demonstrated *in vitro*, and on the possibility of the dosed radioactivity be included in the biosynthesis of other biological molecules and not collected in the mass balance study. As such, the ability to draw conclusions from this study is limited but, at the same time, it is not expected that different experimental conditions could further be pursued. In any case, the biochemistry of the two main considered species (sepiapterin and BH_4) are well known. Also, the CLr of total radioactivity confirmed that the overall renal elimination much lower than the normal GFR. From the remaining PK studies, sepiapterin had terminal half-lives around 4.5 – 5.7h after single dose, and more 3 h at repeated doses (Study PKU-001). BH_4 had terminal half-lives at 3.5- 4.6 hours in Study PKU-001, and around 3.9 - 4.98 h for Study PTC923-MD-005-HV.

The plasma radioactivity rapidly increases after dosing, with a slight drop at 6h before increasing again up to 32 h after dosing, followed by a very slow disappearance with a elimination half-life of 190 h. This is, again, compatible with the inclusion of the dosed radioactivity in the biosynthesis of other biological molecules. It should be stressed out that the observation of radioactivity in the excreta was negligible after 240 h. In the first 24 h, sepiapterin was a minor circulating component and BH_4 one of the most abundant metabolites (together with XH_2). The remaining metabolites were all less relevant in this sample period. On the 0 to 240 hours pooled samples, sepiapterin was again a minor circulating component and seven metabolites were identified, all of them were less than 5% of the total plasma radioactivity. Qualitatively, the metabolite profiles of the 0 to 24 hours and 0 to 240 hours pools were similar to each other. Regarding excreta, unchanged sepiapterin along with pterin accounted for 1.8% of the urine collected radioactivity. M282/1 was the most abundant metabolite accounting for 36%, followed by BH_4, XH_2, and biopterin, accounting for 18.6%, 16.3%, and 14.7% of the urine radioactivity respectively. Five less abundant metabolites were tentatively identified, each accounting for less than 4.5% of the radioactivity in pooled urine from 0 to 72 hours postdose. Regarding faeces, unchanged sepiapterin coeluted with M238/2 and M224/1 and together accounted for 41.3% of the pooled radioactivity. M221/1 and M222/1 (coeluting) were the less abundant metabolites, accounting for 18.3% of the radioactivity in total. Other metabolites tentatively identified were minor, each accounting for less than 6% of the radioactivity in pooled faeces.

Major metabolic pathways included hydrogenation/reduction, dehydrogenation, oxidative deamination, dehydration, oxidation, methylation, and C-C bond side chain cleavage, alone or in combination.

Overall, the observed data is in agreement with the known biosynthesis and metabolism of BH_4. Sepiapterin is rapidly converted to BH_4 *in vivo* by a 2-step reduction primarily mediated by sepiapterin reductase (SR)/carbonyl reductase and dihydrofolate reductase (DHFR) in the pterin salvage pathway.

The drug substance sepiapterin is the synthetic analogue of the natural product sepiapterin, which is formed *in vivo* only as the S-enantiomer. Sepiapterin manufactured by the current manufacturing process has the same stereo configuration as the natural product, controlled by the chirality of the starting material.

BH_4 is the active metabolite and responsible for 9.2% of the total radioactivity in the first 24 h after administration of sepiapterin. This value may be even larger as the conversion of BH_4 into other more stable biopterins is likely during extensive sample processing and enrichment in sample preparation. Being an endogenous compound (as well as the parent administered one), its PK characterization deemed sufficient in the current application.

Dose proportionality and time dependency

Linearity of PK of sepiapterin and BH_4 was studied both after single and multiple doses with and without food. Since no accumulation was observed and the PK seems similar, single and multiple dose data was also combined for further evaluation. In all conditions and for both substances, the increase of C_{max} and AUC with dose was less than proportional. This seems to be more evident for BH_4 for doses above 20 mg/kg after a high-fat meal, and probably for doses higher than 7.5 mg/kg in fasting conditions. Since the elimination half-life is not significantly altered with the increase of dose, this may be mainly due to the absorption process and probably due to the low aqueous solubility.

Data from multiple-dose studies showed no accumulation of both sepiapterin and BH_4. C_{max} and AUC values are similar under SD and MD conditions. No changes in BH_4 elimination $t_{1/2}$ were also observed. Taking all in consideration, no time-dependencies are expected in sepiapterin PK.

Intra- and inter-individual variability

The variability of sepiapterin, a drug with low permeability and high extraction ratio, is expectably high. However, the overall variability in the BH_4 concentrations and PK parameters is low-to medium, as typically observed in metabolites.

Pharmacokinetics in the target population

The PK data obtained in patients showed usually inferior exposures than the ones observed in healthy subjects for the same weight-base doses. When determined, the elimination $t_{1/2}$ are similar between the two populations. This may indicate that the differences may be in the absorption or in the pre-systemic biotransformation. In fact, the popPK model retained the "health status" as a relevant covariate related to the fraction of absorption and the endogenous BH_4 baseline value.

Based on the Exposure-response analysis, a BH_4 AUC > 1764 seems to be needed for a more than 80% probability of ≥ 30% Phe reduction. These AUC values are typically seen for Doses > 40 mg/kg.

Special populations

No studies have been performed in renal and hepatically impaired subjects. The Applicant confirmed that dedicated studies to assess the effect of renal and hepatic impairment on the pharmacokinetics (PK) and safety of sepiapterin and the active metabolite BH4 are still ongoing, and committed to submit the final results via a Type-II variation post-authorisation.

No gender differences were observed in the PK of sepiapterin of BH_4.

The Applicant performed a dedicated study comparing the PK of sepiapterin and BH_4 in Japanese and non-Japanese subjects showing limited differences. The popPK model retained the effect of "being Asian" as a covariate in Vp/F. No dose adjustment for this population is considered necessary.

Weight was shown to be influencing both Volume of distribution and Clearance of BH_4, being retained in the popPK model as a significant covariable in those PK parameters. Dosing of sepiapterin is also based on weight.

No subjects older than 65 were present in the clinical studies, although male or female subjects of any age were considered as an inclusion criterion in the phase III studies. The SmPC proposes the following for the elderly patients: "The safety and efficacy of Sephience in patients 65 years of age and older have not been established. Caution should be exercised when prescribing in patients 65 years of age and older." This is acceptable.

Based on a prospective popPK study developed with adult data and including a maturation function on the CL/F, the Applicant proposed to perform the clinical Phase III studies by dosing with 7.5, 15, 30, and 60 mg/kg for patients with PKU of age group 0 to <6 months, 6 to <12 months, 12 months to <2 years, and ≥2 years, respectively. This was accepted in the PIP. However, during the phase 3 studies only a very limited number of subjects was included with less than 2 years (n = 2) and none with less than 12 months.

The Applicant, following a recent data cut of study PTC923-MD-004-PKU, expanded the data set with a total of 15 participants with <2 years of age, 10 with PK data (4 <1 year and 6 >1 to <2years) and 15 with PD data. These patients were subjected to an age-based dose escalation according to:

- 7.5 mg/kg for subjects 0 to <6 months of age
- 15 mg/kg for subjects 6 to <12 months of age

- 30 mg/kg for subjects 12 months to <2 years of age
- 60 mg/kg for subjects ≥2 years of age

The proposed dosing regimen was based on a prospective population PK model developed using clinical data in healthy adult participants from Study PKU-001 and externally validated with clinical data from participants with primary tetrahydrobiopterin deficiency (PBD) in Study PBD-001, adding a maturation function for predicting the clearance changes in the paediatric population.

When evaluating PK data from sparse and rich sampling from Study PTC923-MD-003-PKU and Study PTC923-MD-004-PKU by age group, it seemed clear that lower BH4 exposures are obtained in participants <2 years of age. As such, the introduction of the maturation function in the prospective popPK model was no longer supported by the available data. Also, an updated popPK model was developed, with the same structure as the prospective model did not require the consideration of the renal and transporter maturation functions utilised to project dose in the prospective model. Although the Applicant argued that the comparable sepiapterin efficacy between participants with PKU <2 years and ≥2 years of age was observed, the conclusions from the updated popPK-PD model was that, at lower doses (20 and 40 mg/kg QD for patients of age ≥2 years, and 7.5, 15, and 30 mg/kg for patients of age < 2 years), comparable but lower percentages of PKU patients could attain the same efficacy targets (greater than 30% Phe reduction, Phe normalization, Phe control, and Phe less than 600 µM). However, more patients would benefit from the treatment across all ages, from neonates to adults, if treated at 60 mg/kg/day. The Applicant was asked to explore this possibility by providing simulations of alternative dose regimens for patients <1 year old and 1-2 years old and presenting them according to the Modelling and simulation questions and answers on presentation of results/predictions of PK analyses in paediatric patients. The Applicant was also asked to discuss if any dosing recommendation below 1 year old would be possible. Since the previous round, the Applicant updated the number of patients < 2 years of age from study PTC923-MD-004-PKU to 33 (previously 15), 8 aged <1 year.

The Applicant elaborated on the new data provided which considers sufficient to support the dose and efficacy/safety of treatment in this population without the need to develop popPK modelling as suggested. Analysis per age group showed a similar response to sepiapterin, with slightly lower results in the younger ages (mainly in the 1-2 year old group). Nevertheless, the results were consistent with results seen in older patients. A similar proportion of patients also achieved the goal serum Phe threshold levels by Week 2. Treated patients had their blood Phe levels under the goal limit for more than 24 months. Of the 33 patients, 8 completed the 26 weeks Dietary Phe Tolerance Assessment, with 7 other still ongoing. Those patients tolerated a similar increase in Phe ingestion at week 26, when compared to older patients, constantly keeping Phe levels below the target goal. Interim results for the 7 patients still ongoing are also discussed, being similar to the ones that already completed the challenge. Out of the 8 patients that completed the Dietary Phe Tolerance Assessment, 3 were aged <1 year with similar results to patients 1-<2 years. Interestingly the change from baseline in term of mg/kg/day was higher in patients <2 years compared to the older ones, perhaps related to the fact that younger patients have more controlled diets and higher margin of progression in terms of tolerance. The Applicant further elaborates on the safety profile of sepiapterin.

The Applicant did not elaborate on the request to further assess possible benefit of a higher dose for children <2 years old based on the popPK model results. As the currently used dose demonstrated consistent decreases in blood Phe levels, maintenance of blood Phe levels below standard goal and tolerance of Phe dietary increases, in line with the results in older patients, this is considered acceptable.

Regarding the older children, there seems to be no clear trend of age dependency of BH_4 exposures with the bodyweight-based dose regimen in patients ≥2 years of age. The proposed dosing in the SmPC is following the

phase III studies recommendations and it is referred that only paediatric patients with PKU, aged 1 to 17 years, have been treated with Sephience in clinical studies.

Pharmacokinetic interactions studies

The Applicant tried to perform the *in vitro* studies in relevant concentration ranges. In some cases, due to the possibility of very high concentration values as defined in the theoretic static models cut-off values, the highest possible concentration to be tested was below those values. This is acceptable. IC_{50} values were considered but, since in general no inhibition was observed, this has limited consequences in the overall conclusions.

Sepiapterin is quickly converted to BH2 by SR and then to BH_4 by DHFR unidirectionally, therefore the conversion/formation of BH_4 following sepiapterin oral administration may be inhibited by the inhibitors of DHFR enzymes. Since no specific studies have been conducted to investigate the effects of these enzyme inhibitors, the SmPC section 4.4 stated that caution is recommended when using such medicinal products while taking sepiapterin. More frequent monitoring of blood Phe concentration may be required because these drugs may inhibit the enzymatic conversion of sepiapterin to BH_4. However, since both SR and CR participate in the initial step of the two-step reduction of sepiapterin, as supported by literature evidence, the inhibition of SR alone is unlikely to completely block this reduction.

Regarding sepiapterin, as a precipitant, no competitive nor time-dependent inhibition was observed for any of the CYP enzymes studied. Sepiapterin was also not an inducer for CYP1A2, CYP2B6 and CYP3A4. Regarding transporters, sepiapterin was an inhibitor of BCRP (IC50 647.7 µM) and ENT1 (IC50 2377 µM). Since, these IC50 are above the cut-off of 0.1× dose/250 ml (i.e., 7.08 µM) the potential clinical DDI between sepiapterin and these transporters substrates could be ruled out. Sepiapterin was also considered a substrate of BCRP. UGT inhibition was not explored which, since no glucuronides of sepiapterin are known, is acceptable.

Regarding BH_4, no studies on the CYP inhibition or induction potential were presented. However, based on the known behaviour of BH_4 from other medicinal products, BH_4 seems not to be an inhibitor or inducer of CYP enzymes. The interaction potential of the major metabolite BH_4 as an inhibitor of human efflux transporters BCRP, BSEP, and MDR1, as well as human uptake transporters MATE1, MATE2-K, OAT1, OAT3, OATP1B1, OATP1B3, OCT1, OCT2, ENT1, and ENT2 was studied in vitro. BH_4 was considered an inhibitor of MDR1 (P-gp) (IC50 992.9 µM), BCRP (IC50 778.4 µM), BSEP and a weak inhibitor for OCT1 (32% at 1500 µM) and OCT2 (23% at 150 µM).

Based on the in vitro data Sepiapterin is a substrate of efflux transporter BCRP and a substrate of ENT2 only at the highest tested concentration. However, Sepiapterin is not a substrate of MDR1 (P-gp), OATP1B1/3, MATEs, OATs, OCT2 and ENT1 transporters.

Based on in vitro studies, BH_4 is a substrate of the efflux transporters BCRP and P-gp. Additionally, BH_4 is a substrate of the MATE2-K uptake transporter, but it is unlikely to be a substrate for the uptake transporters MATE1, OAT1, OATP1B1, OATP1B3, OCT1, and OCT2.

Following the *in vitro* findings, the Applicant performed a DDI study on the effect of sepiapterin as victim and perpetrator at the BCRP level. Curcumin, a known *in vitro* and *in vivo* inhibitor of hBCRP was used for evaluation the sepiapterin victim potential and Rosuvastatin for evaluation the sepiapterin perpetrator potential. In both cases, the effect was tested by co-administration of both drugs and comparison with the administration alone.

The study of sepiapterin as a victim was made with a dose of 20 mg/kg in order to be in the linear part of its PK. This resulted in a small increase of BH_4 exposure-related parameters when sepiapterin is administered with

Curcumin compared with sepiapterin alone. The overall estimated GMRs (expressed as a percentage) (90% CI) for overall C_{max}, AUC0-last, and AUC0-inf of BH_4 were 123.99% (115.32% to 133.31%), 120.49% (113.08% to 128.38%), and 120.04% (112.38% to 128.22%), respectively. However, for the C421.CA genotype the C_{max} and $AUC_{0\text{-}inf}$ of BH_4 were 138.59% (113.99%, 168.51%), 133.36% (108.72%, 163.66%), respectively. This increase is not considered clinically significant, therefore no dose adjustment of Sepiapterin is necessary.

The study of sepiapterin as a perpetrator was made with the highest clinical dose of 60 mg/kg. Results showed that after an oral coadministration of sepiapterin (60 mg/kg) with rosuvastatin, a BCRP substrate, the overall estimated GMRs (expressed as percentage) (90% CI) for C max, AUC 0-last, and AUC 0-inf of rosuvastatin were 112.97% (99.58% to 128.16%), 102.46% (93.26% to 112.57%), and 101.24% (88.84% to 115.36%), respectively. The 90% CI of GMRs were within the no-effect limits of 80% to 125% for AUC 0-last and AUC 0-inf. The upper bound of 90%CI of GMR for C max was only slightly above the 125% limit. This increase is not considered significant.

Although BH_4 was shown to be a substrate and inhibitor of P-gp in vitro, conversion from sepiapterin to BH_4 occurs exclusively intracellularly post absorption. As such, following oral administration of sepiapterin, no interaction is anticipated between BH_4 and P-gp expressed in intestine. Also, previously known data from other medicinal products have shown that BH4 does not influence the PK of the P-gp substrate digoxin. The potential for BH4 inhibiting either the hepatic and renal P-gp is also considered unlikely.

The effect of food was studied in vivo and the results showed that administration of Sepiapterin with food resulted in increased exposure to both sepiapterin and BH_4. Higher increases in exposure were observed after administration with higher-fat meals. BH_4 Cmax increased 2.21-2.26-fold and AUC0-24h increased 2.51-2.84-fold compared with fasting administration. Therefore, the Applicant recommend that Sepiapterin should be taken with food and this is endorsed.

Pharmacodynamics

Sepiapterin (previously known as PTC923 or CNSA-001) is a chemical entity, exogenously synthesized, structurally equivalent version of the biologically produced compound sepiapterin, precursor for the biosynthesis of BH_4 (tetrahydrobiopterin), being developed for the treatment of hyperphenylalaninaemia (HPA) in patients with phenylketonuria (PKU). After administration, sepiapterin is rapidly and extensively converted to BH_4.

Sepiapterin functions in a dual capacity as (1) an endogenous precursor of naturally occurring tetrahydrobiopterin (BH_4), and (2) a pharmacologically active chaperone for phenylalanine hydroxylase (PAH) native state conformation, conferring stability against thermal unfolding and resulting in enhanced activity and prolongation of PAH function. Submitted *in vitro* study (Study PTC923-2023-099) confirmed the combined chaperone activities of BH_4 (from sepiapterin metabolism) after sepiapterin administration however, this dual activity needs to be confirmed *in vivo*. PAH catalyses the conversion of the essential amino acid phenylalanine (Phe) to tyrosine (Tyr) with the help of the cofactor BH_4.

Phe is a predictive pharmacodynamic (PD) biomarker of clinical outcomes in the development of treatments for PKU. Blood Phe concentration was a surrogate primary clinical efficacy endpoint in the pivotal, randomized, controlled, Phase 3 study (PTC923-MD-003-PKU) and in the Phase 2 open-label, active-controlled proof-of-concept study (PKU-002) in subjects with PKU. Data showing that sepiapterin administration was associated with a significant reduction in blood Phe concentration are presented in the overview of efficacy.

In a Phase 1/2 study in subjects with PBD (Study PBD-001), sepiapterin administration for 7 days was associated with a significant reduction in blood Phe concentration. In addition, increases in BH_2 and BH_4 in the CSF were observed in a Phase 1 safety and tolerability, PK, and PD study in healthy volunteers (Study PKU-001).

Given the efficacy of sepiapterin in reducing blood Phe concentration via an increase in BH_4 concentration and a chaperone effect to restore PAH activity, change in blood Tyr concentration and Phe:Tyr ratio was used as exploratory endpoints of sepiapterin efficacy in Study PTC923-MD-003-PKU.

Absolute tyrosine levels remained constant in Study PTC923-MD-003-PKU in part 1 and 2, for both sepiapterin and placebo. The Phe/Tyr ratio in part 1 was also comparable and in part 2 the Phe/Try ratio was reduced when compared to placebo due to decrease of Phe levels, which can be expected. There is no increase in tyrosine levels which could be expected through restoration of PAH activity. Given the sustained and normal tyrosine blood levels at least throughout 26 weeks of sepiapterin therapy, the need for tyrosine monitoring in patients treated with sepiapterin was considered not clinically necessary.

The potential risk of off-target interactions was assessed in vitro using 94 unique off-target assays. There was no inhibition or stimulation greater than 50% observed for any of the receptors studied, indicating that sepiapterin is unlikely to have any significant off-target interactions.

Evaluations of PK/PD relationships for QT prolongation were conducted based on data from healthy adult subjects in Study PTC923-MD-005-HV (Report PTC923-2023-043) and based on pooled data from paediatric and adult patients with PKU and healthy adult subjects in Studies PTC923-MD-005-HV, PTC923-MD-007-HV, PTC923-DDI-101-HV, and PTC923-MD-003- PKU.

Although occasional ECG abnormalities were observed during studies conducted in subjects with PKU, none was considered clinically significant with minimal TEAEs related to ECG findings or any other cardiac disorders.

Two concentration-QT corrected interval (C-QTc) analyses were performed using the linear mixed effect model: one utilizing data from Study PTC923-MD-005-HV (Study PTC923-2023-043) and one using pooled data from 4 clinical studies of sepiapterin (Study PTC923-2023-062).

Based on both analyses, the upper bounds of the 90% CI for the predicted QTcF change from baseline (ΔQTcF) at the C_{max} for the therapeutic dose (60 mg/kg orally) with and without relevant intrinsic and extrinsic factors were below the regulatory threshold of 10 msec, indicating that BH_4, and consequently sepiapterin, does not prolong the QTc interval.

In the SmPC of sapropterin, shortening of the QT interval (-8.32 msec) was observed in a study with a single supratherapeutic dose of 100 mg/kg (5 times the maximum recommended dose). The SmPC includes the warning in section 4.9 (overdose) that this should be taken into consideration in managing patients who have a pre-existing shortened QT interval.

Shortening of the QT interval has been reported in a study with single supratherapeutic dose of sapropterin (100 mg/kg, 5 times the recommended dose).

The two C-QT analysis performed for sepiapterin were primarily based on healthy patients and although simulation scenarios showed similar expected ΔQTcF, the exposure of PKU subjects was lower that healthy subjects (C_{max} of 2.82 ng/mL *vs* 4.66 ng/mL, respectively).

To further establish the effect of sepiapterin in the QT interval, since submission of the MAA, the Applicant has completed Study PTC923-TQT-102-HV that evaluated the supratherapeutic dose of 120 mg/kg in healthy

volunteers. The results of the study indicate that a dose of sepiapterin up to 120 mg/kg/day and the resulting BH4 exposure had minimal to no effect on either the shortening or prolongation of the QT interval.

Sepiapterin is converted to BH_4 by SR and DHFR and therefore, medicines able to modulate both enzymes could potentially alter the efficacy of sepiapterin.

Although there is no clinical data on these possible interactions, the Applicant has addressed that issue in sections 4.4 and 4.5 of the SmPC.

Some published studies have identified specific PAH mutations correlated to the presence/absence of BH_4 responsiveness.

Although understanding the genotype-phenotype relationship of different PAH variants with respect to BH4 responsiveness could be useful, the Applicant stated that the high genetic diversity of PAH variants and the limited number of participants treated with sepiapterin makes it not possible to draw true meaningful genotype-phenotype relationship regarding sepiapterin response and any given genotype(s).

Nevertheless, the Applicant believes that the dual mechanism of action of sepiapterin and the nonclinical and clinical data support the efficacy of sepiapterin across a range of PKU genotypes and phenotypes, including those known to be nonresponsive to sapropterin. In support of these arguments are the theoretical assumptions based on the mechanism of action; the non-clinical data showing that sepiapterin increases the activity of PAH mutant proteins that are not BH-4 responsive and the clinical data showing patients that where unresponsive to sapropterin and responded to sepiapterin.

Pharmacokinetic/Pharmacodynamic (PK/PD) modelling of Phe levels after oral sepiapterin administration in patients with PKU was developed based on data from the Phase 3 study PTC923-MD-003-PKU (Report PTC923-2021-022). An exposure-efficacy analysis with logistic regression was conducted for Phe reduction in patients with PKU following sepiapterin treatment using data from the Phase 3 Study PTC923-MD-003-PKU and BH_4 exposure from Bayesian simulation based on the population PK and plasma concentrations observed in Studies PTC923-MD-003-PKU and PTC923-MD-004-PKU.

The study primary endpoint was met, with a statistically significant difference in reduction in blood Phe levels in the sepiapterin treatment arm *versus* placebo following 6 weeks of therapy in PKU participants $\geqslant$2 years of age. By Week 6, mean blood Phe levels decreased significantly in participants who received sepiapterin. In contrast, Phe levels remained relatively unchanged in the placebo arm.

A statistically significant difference in the mean change in blood Phe levels was achieved from Week 1 and 2 and was sustained through Weeks 5 and 6. 89.8% of participants who received sepiapterin achieved a ≥30% reduction in blood Phe levels compared with 10.2% who received placebo.

For those with a baseline Phe level ≥600 µmol/L, 92.9% of participants who received sepiapterin achieved Phe levels <600 µmol/L in Part 2, compared with 30.0% of participants who received placebo.

The vast majority (84.1%) of participants treated with sepiapterin achieved the American College of Medical Genetics and Genomics (ACMG)-recommended blood Phe level of <360 µmol/L, with 22% of participants reaching normalized blood Phe levels of <120 µmol/L. In contrast, after placebo, only 9.3% of participants reached blood Phe levels <360 µmol/L, and no participants achieved normalized Phe levels.

The efficacy of BH_4 on Phe level in 154 patients with PKU enrolled in Study PTC023-MD-003PKU was evaluated using simulated BH_4 exposure levels (AUC, minimum concentration [C_{min}], and C_{max}) and blood Phe level as continuous efficacy measures. Based on the visual predictive check (VPC) plots, the model was found to

adequately predict the median response in patients with PKU indicating the suitability of the final PK/PD model for predicting the efficacy of sepiapterin.

The Applicant has supported the dose selection with data from the first-in-human healthy volunteer Study PKU-001 demonstrating that dose-related increases in plasma sepiapterin and BH_4 were observed and increases in plasma BH_4 with sepiapterin administration were greater than those obtained with sapropterin administered at the same dose.

On the Phase 2 head-to-head study PKU-002 (with doses of 20 mg/kg/day and 60 mg/kg/day) in patients with PKU, sepiapterin provided significantly greater, dose-related reduction in blood Phe concentration in patients with PKU compared with sapropterin.

On the Phase 3, placebo-controlled, double-blind, global, PTC923-MD-003-PKU study, there was evidence of clinically meaningful effect in broad PKU population of children and adults with varying severity of disease. Six weeks of treatment up to a maximum dose of 60 mg/kg/day sepiapterin produced statistically significant, clinically meaningful benefit relative to placebo across multiple efficacy endpoints.

2.6.4. Conclusions on clinical pharmacology

Regarding the **clinical pharmacokinetics,** it can be considered as sufficiently well described on most topics.

The initially presented PK, efficacy and safety data were insufficient to support an indication in children below 2 years of age. The Applicant was requested to further discuss and justify an extrapolation of efficacy and safety from adults to children < 2 years old. The available data showed a lower exposure of BH4 for children <2 years old and the popPK model suggested that all patients could benefit from a fixed 60 mg/kg QD dose regimen. The Applicant was asked to explore this possibility by providing simulations of alternative dose regimens for patients <1 year old and 1-2 years old and presenting them according to the Modelling and simulation questions and answers on presentation of results/predictions of PK analyses in paediatric patients. The Applicant was also asked to discuss if any dosing recommendation below 1 year old would be possible. In the response, the Applicant updated the number of patients < 2 years of age from study PTC923-MD-004-PKU to 33 (previously 15), 8 aged <1 year. Based on the new data provided, the Applicant considered that there is no need to develop a new popPK model as the data is sufficient to support the dose and efficacy/safety of the treatment in this age group. The Applicant did not elaborate on the request to further assess possible benefit of a higher dose for children <2 years old based on the popPK model results. As the currently used dose demonstrated consistent decreases in blood Phe levels, maintenance of blood Phe levels below standard goal and tolerance of Phe dietary increases, in line with the results in older patients, this is considered acceptable.

Data about special populations, such as renal and/or hepatic impaired subjects, are missing. The Applicant confirmed that the dedicated studies to assess the effect of renal and hepatic impairment on the pharmacokinetics (PK) and safety of sepiapterin and the active metabolite BH4 are still ongoing, and committed to submit the final results via a Type-II variation post-authorisation.

The **clinical pharmacodynamic** section of this application is based on data from the Phase 1, Phase 2 and Phase 3 studies. Generally, the characterization of the pharmacodynamics of sepiapterin can be considered appropriate.

Since the primary outcome of the main study is a PD marker (blood Phe), the primary PD effect is described, well characterized and discussed in detail in the Clinical Efficacy section of this report.

2.6.5. Clinical efficacy

Study Identifier	Study Design	Dose/Number of Subjects	Endpoints	Statistical Methods
Pivotal Study				
PTC923-MD-003-PKU (completed) 34 sites 13 countries	Phase 3, multicenter, 2-part, double-blind, placebo-controlled, randomized Part 1: 14-day open-label sepiapterin assessment of responsiveness Part 2: randomization (1:1) to either 6 weeks sepiapterin or placebo (stratification based on baseline blood Phe and reduction in blood Phe)	Part 1: N=156 (Subjects in Part 1 only: N=47) 7.5 mg/kg sepiapterin (0 to <6 months of age), 15 mg/kg (6 to <12 months of age), 30 mg/kg (12 months to <2 years of age), 60 mg/kg (⩾ 2 years of age) for 14 days starting on Day 1. Part 2: N=110 (56 in sepiapterin arm and 54 in placebo arm) Sepiapterin: 20 mg/kg daily for Days 1 to 14 (i.e., Weeks 1 and 2), 40 mg/kg daily for Days 15 to 28 (i.e., Weeks 3 and 4), 60 mg/kg daily for Days 29 to 42 (i.e., Weeks 5 and 6). Matched dose equivalent placebo Overall enrolment: ⩾1 to <2 years; n=0 ⩾2 to <6 years; n=10 ⩾6 to <12 years; n=29 ⩾12 to <18 years; n=33 ⩾18 years; n=38	**Primary endpoint** • Mean change in blood Phe concentration from baseline to Week 5 and Week 6 (i.e., the average of each respective treatment dose 2-week period of double-blind treatment) **Secondary endpoints** • The proportion of subjects with baseline Phe concentration ≥ 600 µmol/L who achieved Phe concentration <600 µmol/L at the end of the double-blind treatment period. • Proportion of subjects with baseline Phe concentration ≥360 µmol/L who achieved a Phe concentration <360 µmol/L at the end of the doubleblind treatment period. • Mean change and percent change from baseline in blood Phe concentration at each dose level • PK concentrations and parameters of sepiapterin and BH_4	**Primary endpoint:** Treatment difference and corresponding 95% CI between sepiapterin and placebo in the mean change from baseline to Weeks 5 and 6 were estimated using a MMRM method. **Secondary endpoint:** Odds ratio of sepiapterin versus placebo along with the 95% CI were estimated using a chi-square or Cochran-Mantel-Haenszel test.
Additional Studies				
PKU-002 (Completed) 4 sites 3 sites in Australia and 1 site in Europe	Phase 2, randomized, double crossover, open-label, active-controlled study of sepiapterin	N=24 (all ⩾18 years) All subjects received 7 days of 20 mg/kg sepiapterin, 60 mg/kg sepiapterin, and 20 mg/kg sapropterin	**Primary endpoint:** Absolute mean and % mean change in plasma Phe concentrations from baseline	Change from baseline was estimated using a MMRM model with fixed effects for overall study baseline blood Phe, treatment group, sequence, period, and a random patient effect within each sequence, with a first order autoregressive AR (1) covariance structure. Pairwise comparisons in changes from baseline between each treatment

				was performed by calculating the least squares means with Dunnett's method of adjustment for multiple comparisons.
PTC923-MD-004-PKU (Ongoing) 33 sites 15 countries	Phase 3: open-label, safety, efficacy, quality of life	N=169[a] (~200 planned) 7.5, 15, 30, 60 mg/kg for a minimum of 1 year Overall enrolment: 0 to < 6 months; n =5 ⩾1 to <2 years; n=9 ⩾2 to <6 years; n=19 ⩾6 to <12 years; n=32 ⩾12 to <18 years; n=44 ⩾18 years; n=60	**Primary endpoint:** Change from baseline in dietary Phe/protein consumption **Secondary endpoint:** Sepiapterin and BH_4 concentrations in plasma	**Primary endpoint** Change in daily dietary Phe consumption from baseline during the 26-week Phe tolerance assessment period was fitted by a MMRM model based on the Dietary Phe Tolerance Analysis Set, with the fixed effects as baseline Phe consumption and week. Subject were included as random effect. The LSM estimate for the average change at Week 26 and 95% CI was also presented.

The clinical programme for this MAA includes 3 main clinical studies:

- a completed, open-label, active controlled study phase 2 Study (PKU-002) in 24 subjects;
- a completed, Phase 3, randomized, placebo-controlled pivotal study (PTC923-MD-003-PKU) in 157 subjects;
- an ongoing long term, open-label, safety and efficacy study (PTC923-MD-004-PKU) in 169 subjects.

The proposed indication is: "Sephience is indicated for the treatment of hyperphenylalaninaemia (HPA) in adult and paediatric subjects with phenylketonuria (PKU)."

A total of 246 subjects with PKU have been treated. This is acceptable as regard to the small number of affected subjects in the whole population.

2.6.5.1. Dose response study(ies)

Study PKU-002 (completed) was a Phase 2, randomized, multicenter, double-crossover, open label, active-controlled, study in adult subjects with PKU. All 24 participants screened were deemed eligible and were randomised in Study PKU-002 in 6 sequence groups of 4 subjects per group. Each sequence group was randomized to receive sepiapterin 60 mg/kg/day, sepiapterin 20 mg/kg/day, and sapropterin 20 mg/kg/day, in random order over a period of 7 days, with a 7-day washout period between each treatment sequence.

The primary objective was to assess preliminary efficacy of sepiapterin in reducing blood Phe levels at Day 3, 5 and 7 versus Day 1 for each period of treatment. A secondary objective of this study was to establish the dose to be used in future Phase 3 studies in PKU and to assess safety and tolerability of 2 doses of CNSA-001 in PKU patients.

Dose selection for the pivotal Phase 3 was based on pharmacokinetics, pharmacodynamics/biomarker, efficacy, and safety data from study PKU-002 in adult subjects with PKU.

This study was a proof of concept, no formal sample size calculation has been performed and the study was not powered to show statistic difference in blood Phe concentration between arms.

Results showed that treatment with sepiapterin 20 mg/kg and 60 mg/kg/day resulted in a significant dose-dependent decrease in blood Phe concentration relative to baseline.

Pairwise comparisons of LSM blood Phe concentration reductions for all treatments favoured sepiapterin 60 mg/kg/day.

In subjects with classical PKU (11/25 [45.8%]), treatment with sepiapterin (60 mg/kg/day) resulted in a significant decrease in blood Phe concentration relative to baseline (p=0.0287).

Since this is an exploratory study for which no formal sample size calculation was performed and the response of subjects to BH_4 was unknown, comparisons between the sepiapterin and sapropterin arms are not relevant and no valid conclusion on the effect difference between the arms can be drawn from this study. Additionally, several endpoints have been defined as primary endpoints and adjustment for multiple comparison has not been considered across endpoints leading to type I error inflation and multiplicity issues. Considering the above-mentioned limitations, only descriptive data of this study with no reference to inferential statistics and p-values is to be included in the SmPC.

Table 13. Summary of Efficacy for Study PKU-002

Title: A Phase 2, Randomized, Double Crossover, Open-Label, Active Controlled Proof-of-Concept Study of CNSA-001 in Phenylketonuria Patients		
Study identifier	PKU-002 ANZCTR number: ACTRN12618001031257	
Design	This was a Phase 2, randomized, double crossover, open-label, active-controlled proof of concept study of CNSA-001 (sepiapterin) in patients with PKU. The study consisted of 6 sequence groups of 4 patients per group for a total of 24 patients. Each sequence group was randomized to receive 7-day treatments of sepiapterin 60 mg/kg/day, sepiapterin 20 mg/kg/day, and sapropterin (BH_4 or Kuvan) 20 mg/kg/day, in random order. A 7-day washout separated each treatment.	
	Duration of main phase: Duration of Run-in phase:	5 weeks (including treatment and washout periods) 7-day pre study period
Hypothesis	Superiority of sepiapterin versus sapropterin	
Treatments groups	Group A	Sepiapterin 20mg/kg/day; 7 days; 24 randomized
	Group B	Sepiapterin 60 mg/kg/day; 7 days; 24 randomized
	Group C	Sapropterin 20 mg/kg/day; 7 days; 24 randomized
Endpoints and definitions	Primary endpoint	Absolute mean and % mean change in plasma Phe concentrations from baseline
	Secondary endpoints	Safety and tolerability including the assessment of physical examinations, ECGs, vital signs, clinical laboratory results, and adverse events
Database lock	24 July 2019	
Results and Analysis		

Analysis description	**Primary Analysis**			
Analysis population and time point description	Efficacy population (all patients who were randomized, received any amount of sepiapterin or sapropterin, completed at least 1 treatment period within their randomized sequence group, had available pre-dose Phe concentrations from at least 1 treatment period Day 1 (pre-dose) and Day 3, Day 5, or Day 7 postdose and was based on the treatment sequence to which the patient was randomized) Mean blood Phe concentration for Day 3, Day 5, and Day 7 (i.e., weekly mean analysis) for each treatment period versus Day 1 pre-dose concentration			
Descriptive statistics and estimate variability		Efficacy Population (Weekly mean analysis)		
	Treatment group	Sepiapterin (20 mg/kg/day)	Sepiapterin (60 mg/kg/day)	Sapropterin (20 mg/kg/day)
	Number of subjects	24	24	24
	Actual change from baseline in blood Phe concentration (mean)	-143.7	-210.1	-90.8
	Standard deviation	224.79	235.78	226.85
	% Change from baseline in blood Phe concentration	-17.4	-29.3	-16.8
	Standard deviation	54.07	37.69	39.09
Effect estimate per comparison	Primary endpoint	Comparison groups Sepiapterin 20 mg/kg/day versus sapropterin 20 mg/kg/day	Sepiapterin (20 mg/kg/day) versus sapropterin (20 mg/kg/day)	
		Pairwise comparison LS Mean/difference	-55.42	
		SE	38.95	
		p value	0.2740	
	Primary endpoint	Comparison groups Sepiapterin 60 mg/kg/day versus sapropterin 20 mg/kg/day	Sepiapterin (60 mg/kg/day) versus sapropterin (20 mg/kg/day)	
		Pairwise comparison LS Mean/difference	-114.91	
		SE	38.98	
		p value	0.0098	
	Primary endpoint	Comparison groups Sepiapterin 20mg/kg/day versus sepiapterin 60 mg/kg/day	Sepiapterin (20 mg/kg/day) versus Sepiapterin (60 mg/kg/day)	

		Pairwise comparison LS Mean/difference	59.49
		SE	39.04
		p value	0.1349
Analysis description	**Secondary analysis (pre-specified)** • Blood Phe by baseline disease severity (i.e., blood Phe concentrations < 600 µmol/L, 600 to 900 µmol/L, 900 to 1200 µmol/L, > 1200 µmol/L) • Percent of patients with an overall reduction (any reduction in blood Phe concentration) and up to 10%, 20% and 30% reductions in blood Phe by baseline disease severity (i.e., blood Phe concentrations < 600 µmol/L, 600 to 900 µmol/L, 900 to 1200 µmol/L, > 1200 µmol/L) • Percent of patients with blood Phe concentrations in acceptable treatment range of 120 to 360 µmol/L • Percent of patients with normalized blood Phe concentrations defined as Phe < 120 µmol/L • Blood Phe reduction in Classical PKU patients		
Descriptive statistics and estimate variability		Change from Baseline in Plasma Phe by Disease Severity	
	Treatment group	Sepiapterin (20 mg/kg/day)	Sepiapterin (60 mg/kg/day)	sapropterin (20 mg/kg/day)
	Baseline Phe <600 µmol/L Number of subjects	12	9	8
	Actual change from baseline in blood Phe concentration (mean)	-88.6	-214.3	-134.9
	Standard deviation	204.77	188.97	178.23
	% Change from baseline in blood Phe concentration	15.3	-46.4	-34.1
	Standard deviation	146.09	34.47	41.77
Descriptive statistics and estimate variability		Change from Baseline in Plasma Phe by Disease Severity	
	Treatment group	Sepiapterin (20 mg/kg/day)	Sepiapterin (60 mg/kg/day)	sapropterin (20 mg/kg/day)
	Baseline Phe 600 to 900 µmol/L Number of subjects	3	6	8
	Actual change from baseline in	-158.3	-214.2	-222.1

	blood Phe concentration (mean)			
	Standard deviation	412.61	348.42	307.61
	% Change from baseline in blood Phe concentration	-20.8	-27.1	-32.7
	Standard deviation	53.8	44.26	42.72
		Change from Baseline in Plasma Phe by Disease Severity		
Descriptive statistics and estimate variability	Treatment group	Sepiapterin (20 mg/kg/day)	Sepiapterin (60 mg/kg/day)	sapropterin (20 mg/kg/day)
	Baseline Phe 900 to 1200 µmol/L Number of subjects	6	7	5
	Actual change from baseline in blood Phe concentration (mean)	-167.8	-255.1	-45.0
	Standard deviation	328.49	379.36	212.32
	% Change from baseline in blood Phe concentration	-15.4	-27.5	-4.7
	Standard deviation	33.33	40.36	19.89
		Change from Baseline in Plasma Phe by Disease Severity		
Descriptive statistics and estimate variability	Treatment group	Sepiapterin (20 mg/kg/day)	Sepiapterin (60 mg/kg/day)	sapropterin (20 mg/kg/day)
	Baseline Phe > 1200 µmol/L Number of subjects	3	2	3
	Actual change from baseline in blood Phe concentration (mean)	60.0	-195.0	173.3

	Standard deviation	615.06				106.07				228.11			
	% Change from baseline in blood Phe concentration	6.7				-12.6				11.1			
	Standard deviation	48.84				7.97				14.51			
Descriptive statistics and estimate variability		Proportion of Patients with Reduction in Weekly Mean DBS Phenylalanine Levels by Disease Severity											
	Treatment group	Sepiapterin (20 mg/kg/day)				Sepiapterin (60 mg/kg/day)				sapropterin (20 mg/kg/day)			
		Reduction in Plasma Phe Concentration (%)											
		All	⩽10	⩾20	⩾30	All	⩽10	⩾20	⩾30	All	⩽10	⩾20	⩾30
	Baseline Phe < 600 µmol/L Number of subjects	10	9	7	6	8	6	5	5	8	7	7	6
	% subjects	41.7	37.5	29.2	25.0	33.3	25.0	20.8	20.8	33.3	29.2	29.2	25.0
	Baseline Phe 600 to 900 µmol/L Number of subjects	3	2	2	1	7	6	5	4	4	3	2	2
	% subjects	12.5	8.3	8.3	4.2	29.2	25.0	20.8	16.7	16.7	12.5	8.3	8.3
	Baseline Phe 900 to 1200 µmol/L Number of subjects	4	3	2	1	3	1	1	1	4	3	1	0
	% subjects	16.7	12.5	8.3	4.2	12.5	4.2	4.2	4.2	16.7	12.5	4.2	0
	Baseline Phe > 1200 µmol/L Number of subjects	2	2	1	0	2	1	1	0	0	0	0	0
	% subjects	8.3	8.3	4.2	0	8.3	4.2	4.2	0	0	0	0	0
	Total Number of subjects	19	16	12	8	20	14	12	10	16	13	10	8
	% subjects	79.2	66.7	50	33.3	83.3	58.3	50	41.7	66.7	54.2	41.7	33.3
		Proportion of Patients with Normalized Weekly Mean Phe Concentration and Acceptable Treatment Range											
		Sepiapterin				Sepiapterin				sapropterin			

		(20 mg/kg/day)	(60 mg/kg/day)	(20 mg/kg/day)
Descriptive statistics and estimate variability	**Acceptable treatment range (120 to 360 µmol/L)** Number of subjects	8	9	9
	% subjects	33.3	37.5	37.5
	Normalized Phe concentration (<120 µmol/L) Number of subjects	3	3	1
	% subjects	12.5	12.5	4.2
Analysis description	**Secondary analysis (Ad hoc analysis)**			
		Change from Baseline in Plasma Phe in Classical PKU Patients (weekly mean analysis)		
		Sepiapterin 20mg/kg/day	Sepiapterin 60mg/kg/day	Sapropterin 20 mg/kg/day
Descriptive statistics and estimate variability	Number of subjects	11	11	11
	Actual change from baseline in blood Phe concentration (mean)	-75.5	-146.0	10.7
	Standard deviation	201.84	187.67	221.61
	% Change from baseline in blood Phe concentration	-11.2	-17.8	-2.0
	Standard deviation	24.35	26.73	39.23
	Ad hoc analysis in classical PKU	Comparison groups Sepiapterin 20 mg/kg/day versus sapropterin 20 mg/kg/day		Sepiapterin (20 mg/kg/day) versus sapropterin (20 mg/kg/day)
		Pairwise comparison LS Mean/difference		-68.70
		SE		60.35
		p value		0.4336

	Ad hoc analysis in classical PKU	Comparison groups Sepiapterin 60 mg/kg/day versus sapropterin 20 mg/kg/day	Sepiapterin (60 mg/kg/day) versus sapropterin (20 mg/kg/day)
		Pairwise comparison LS Mean/difference	-147.96
		SE	63.00
		p value	0.0566
	Ad hoc analysis in classical PKU	Comparison groups sepiapterin 20 mg/kg/day versus sepiapterin 60 mg/kg/day	Sepiapterin (20 mg/kg/day) versus sepiapterin (60 mg/kg/day)
		Pairwise comparison LS Mean/difference	79.26
		SE	62.12
		p value	0.2192

Abbreviations: D, day; ECG, electrocardiogram; LS, least squares; M, month; Phe, phenylalanine; PKU, phenylketonuria; QOL, quality of life; SD, standard deviation; TEAE, treatment emergent adverse event

Changes in dietary Phe intake relative to baseline were greater in subjects who received either sepiapterin 20 mg/kg/day or sapropterin 20 mg/kg/day. The Applicant has clarified that these discrepancies were within the normal variance for considering a stable diet. Also due to the nature of the crossover design, each patient was exposed to different levels of sepiapterin throughout the study, with the diet having been controlled for each patient. A possible effect of treatment sequence in each patient in the response to treatment was excluded. Furthermore, an analysis excluding those participants who had a ≥20% change relative to baseline in dietary Phe intake throughout the study was conducted, with consistent results compared to the original analysis.

None were previously treated with sapropterin as sapropterin dihydrochloride was not accessible in the countries in which participants were enrolled, Australia and Georgia.

2.6.5.2. Main study(ies)

PTC923MD-003-PKU

The pivotal evidence comes from the results of a double-blind placebo controlled multicentre study in patients of all age with PKU.

The study consisted of 2 parts: an open label phase that aimed to assess the responsiveness of subjects to sepiapterin followed by a double-blind randomized placebo-controlled phase in subjects who responded.

Subjects who experienced a ≥ 15% reduction of blood Phe at the end of part 1 were considered responders and eligible for the part 2. Although not being a recognised definition for treatment responsiveness this cut-off was used with the purpose to create an enriched population for the placebo-controlled part of the pivotal study. After completion of Part 1, and following a minimum 14-day washout period, eligible subjects were randomized in Part 2, excepted subjects under the age of 2 years who experienced a ≥ 15% reduction of blood Phe who

were offered the option to enrol directly into the open label extension of the study as it was considered not ethical to expose young responder subjects to placebo for 6 weeks.

Methods

- **Study Participants**

The study inclusion criteria were developed as the broadest possible to reflect the wider PKU population (male and female participants of all ages with a confirmed clinical diagnosis of PKU), while excluding participants who may be at risk from participation or may confound data interpretation (such as low glomerular filtration rate, BH_4-deficient participants, etc).

Participants were required to have a clinical diagnosis of PKU with HPA documented by past medical history of at least 2 blood Phe measurements ≥600 µmol/L.

Another inclusion criterion specified participants must had blood Phe levels ≥360 µmol/L during screening, or when taking the average of the 3 most recent Phe levels from the participant's medical history (inclusive of the screening value).

Participants with biochemically diagnosed classical PKU (i.e., blood Phe birth levels ≥1200 µmol/L and/or historical evidence of Phe concentrations ≥1200 µmol/L in their medical history) were eligible, but enrolment was capped at 20% of the total study population.

- **Treatments**

Part 1

All participants received sepiapterin for 14 days starting on Day 1:

- 7.5 mg/kg for participants 0 to <6 months of age,
- 15 mg/kg for participants 6 to <12 months of age,
- 30 mg/kg for participants 12 months to <2 years of age,
- 60 mg/kg for participants ≥2 years of age.

The dosage was chosen based on the results of the phase 2 study on adult subjects. There was no dedicated dose finding study in paediatric subjects. A popPK study using sparse and rich sampling from Study PTC923-MD-003-PKU and Study PTC923-MD-004-PKU by age group confirmed a lower exposure for patients <2 years and suggested that all patients would benefit form a fixed 60 mg/kg QD dosage regime.

Part 2

Participants were randomized to receive either sepiapterin or placebo for 42 days (6 weeks).

Participants randomized to the sepiapterin treatment arm received sepiapterin 20 mg/kg daily for Days 1 to 14 (i.e., Weeks 1 and 2), then sepiapterin 40 mg/kg daily for Days 15 to 28 (i.e., Weeks 3 and 4), then sepiapterin 60 mg/kg daily for Days 29 to 42 (i.e., Weeks 5 and 6). As dosing is weight based and to maintain the blind, participants randomized to placebo received equivalent quantities of placebo to match the 20 to 40 to 60 mg/kg dose escalation of those randomized to sepiapterin.

A forced dose escalation was proposed in part 2 of the study when subjects had been previously treated with 60 mg/kg during part 1, which is also the starting dose proposed in the SmPC. Updated data from the ongoing

study including 65 naïve patients who started sepiapterin without dose escalation demonstrated that rhe proposed starting dose of 60 mg/kg is acceptable.

- **Objectives**

The primary objective is to evaluate the efficacy of sepiapterin in reducing blood Phe levels in participants with PKU as measured by mean change in blood Phe levels from baseline to Weeks 5 and 6 (i.e., the average of each respective treatment dose 2-week period of double-blind treatment).

- **Outcomes/endpoints**

Primary endpoint

- Mean change in blood Phe levels from baseline to Weeks 5 and 6 (average over a 2-week period) in the Part 2 double-blind phase.

Secondary Endpoints

- Proportion of participants with baseline Phe levels ≥600 µmol/L who achieve Phe levels <600 µmol/L at the end of the double-blind treatment period
- Proportion of participants with baseline Phe levels ≥360 µmol/L who achieve Phe levels <360 µmol/L at the end of the double-blind treatment period
- Mean change and percent change from baseline in blood Phe levels at each dose level
- PK concentrations and parameters of sepiapterin and BH_4
- Severity and number of TEAEs, clinical laboratory tests, vital signs, physical examinations, and ECGs

- **Sample size**

In Part 1, 187 participants were screened and 157 participants were enrolled to receive sepiapterin.

110 patients were randomized in Part 2, to receive either sepiapterin (n=56) or placebo (n=54) treatment during Part 2.

- **Randomisation and Blinding (masking)**

In Part 1 all patients received open-label sepiapterin.

In Part 2 patients were randomised to receive either sepiapterin or placebo. Increments of sepiapterin were simulated in the placebo arm with increase in the placebo.

- **Statistical methods**

The following datasets were defined for this study:

- **Full Analysis Set (FAS)**: All participants who were randomized and took at least 1 dose of double-blind study drug in Part 2 were included in the FAS. Participants were analysed according to their randomized treatment. All efficacy analyses were based on the FAS.
  - The **primary analysis population** was defined for the stratum of participants with mean percent reduction in blood Phe levels of ≥30% during Part 1.
- **Per-Protocol (PP) Analysis Set**: All participants in the FAS who met the study eligibility requirements and had no major protocol deviations that could affect the validity of the efficacy measurements. This was

a subset of the FAS. The criteria for inclusion in the PP Analysis Set were finalized prior to study unblinding. Participants who met the following criteria were excluded from the PP population:

- Received study treatment different from the randomized treatment throughout Part 2
- Did not have a valid blood Phe at baseline in Part 2, or a valid blood Phe at Day 42 within the allowed analysis visit window
- Had significant noncompliance to study drug administration
- Had significant inclusion or exclusion criteria violations
- Had major protocol deviations (e.g., diet modification) that may impact effectiveness of study treatment

For the primary efficacy endpoint, a gatekeeping procedure was used to control the family-wise error rate, as follows:

- The stratum of participants ≥2 years of age with mean percent reduction in Phe levels of ≥30% during Part 1 was tested at the significance level of 0.05 (2-sided). If $p<0.05$, then the study would be declared positive in this stratum.
- Only if the stratum of participants ≥2 years of age with mean percent reduction in Phe levels of ≥30% during Part 1 was statistically significant at the 0.05 level, would the overall study with all participants ≥2 years of age with a mean percent reduction in Phe levels of ≥15% during Part 1 be tested, also at the 0.05 significance level.

For the primary endpoint of reduction in blood Phe in the primary analysis population, a mixed model repeated measures (MMRM) was fitted using the available blood Phe data. The response variable was the change from baseline in blood Phe measured at postbaseline assessments for each participant. The model included fixed effects for treatment, baseline blood Phe, baseline Phe stratum (<600 or ≥600 µmol/L), visit (categorical), and visit-by-treatment interaction. In addition, participant was included as random effect. An unstructured within-participant covariance structure was assumed. The least squares (LS) mean estimate for the average change at Part 2 Weeks 5 and 6 was used to perform treatment group comparisons. Two sensitivity analyses incorporating either only baseline blood Phe or only baseline Phe stratum as covariates in the MMRM model were conducted. These analyses were performed on participants who had a ≥30% reduction blood Phe in Part 1 of the study and on the FAS. The findings were consistent with the results of the primary analysis conducted using the original MMRM model**.**

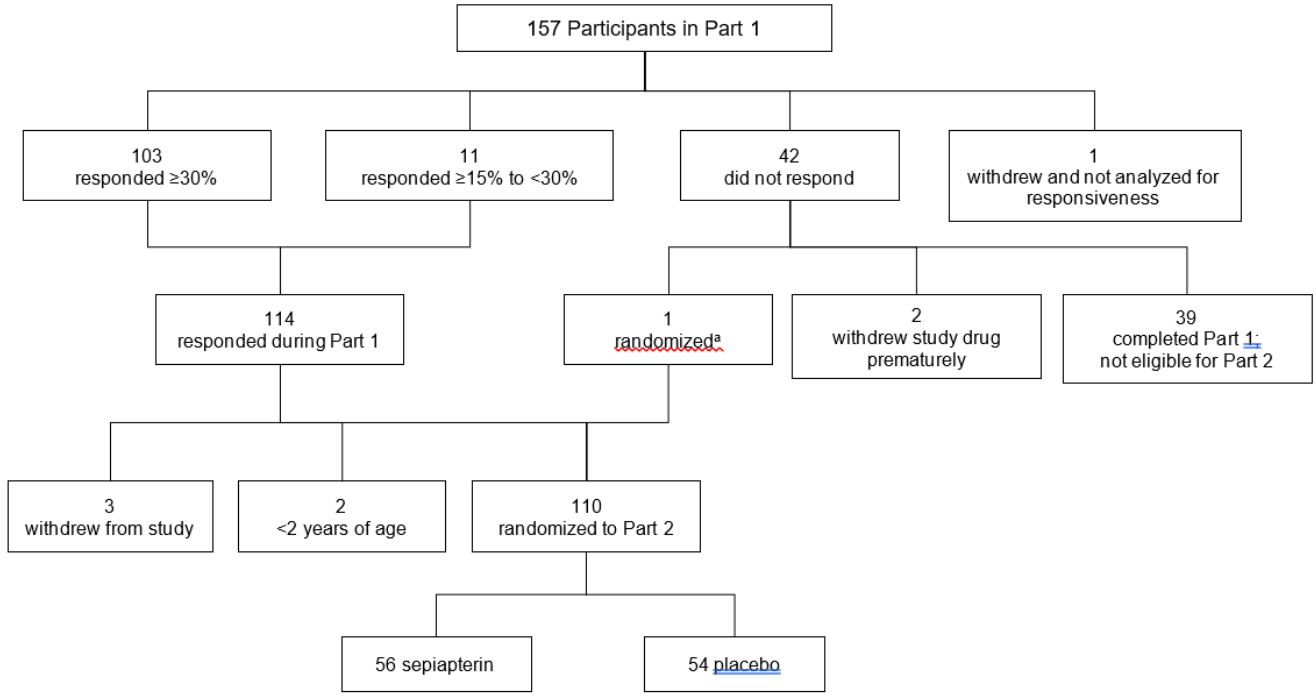
The LS means, treatment effect estimate, confidence interval (CI), and 2-sided p value were presented. Corresponding LS means from the same MMRM model were used to estimate the treatment effects at each visit week.

For the primary endpoint of reduction in blood Phe levels for all participants, the same MMRM model used for the stratum of participants with mean percent reduction in blood Phe levels of ≥30% during Part 1 was used with the addition of a fixed effect for Phe reduction randomization strata (≥15% or ≥30%).

Results

- **Participant flow**

Figure 10. Study disposition



[a] One participant was randomized to Part 2 (sepiapterin group) with a <15% reduction in blood Phe level. The randomization occurred based on the results of 2 of the 3 posttreatment blood Phe levels; when the third value became available, the average resulted in a <15% reduction. After discussion between the investigator and the sponsor, the participant remained in the study.
Note: Participants <2 years of age who were identified as "responsive" during Part 1 continued directly into the long-term safety Study PTC923-MD-004-PKU.

- **Recruitment**

The study enrolled first participant in 30 September 2021 and the last participant completed the trial in 03 April 2023.

- **Conduct of the study**

There were 4 amendments to the original protocol (Version 1.0 dated 23 March 2021) for Study PTC923-MD-003-PKU that included as major modifications:

- Dose update to detail age-dependent dosing
- Update to blood samples to be collected
- Definition Phe responsiveness

- **Baseline data**

Table 14. Demographics and Baseline Characteristics (Safety Analysis Set)

	Subjects in Part 1 Only (N=47)	Randomized and Treated Subjects in Part 2 (FAS)			Overall Treated Subjects (N=157)
		Sepiapterin (N=56)	Placebo (N=54)	Overall (N=110)	
Age at baseline, years					
Median (min, max)	15.0 (1, 61)	13.0 (2, 47)	15.0 (4, 54)	14.0 (2, 54)	14.0 (1, 61)
Age category, n (%)					
≥1 year to <2 years	3 (6.4)	0	0	0	3 (1.9)
≥2 years to <6 years	5 (10.6)	7 (12.5)	3 (5.6)	10 (9.1)	15 (9.6)
≥6 years to <12 years	11 (23.4)	17 (30.4)	12 (22.2)	29 (26.4)	40 (25.5)
≥12 years to <18 years	10 (21.3)	14 (25.0)	19 (35.2)	33 (30.0)	43 (27.4)
<18 years	29 (61.7)	38 (67.9)	34 (63.0)	72 (65.5)	101 (64.3)
≥18 years	18 (38.3)	18 (32.1)	20 (37.0)	38 (34.5)	56 (35.7)
Sex, n (%)					
Male	28 (59.6)	30 (53.6)	27 (50.0)	57 (51.8)	85 (54.1)
Female	19 (40.4)	26 (45.4)	27 (50.0)	53 (48.2)	72 (45.9)
Ethnicity, n (%)					
Hispanic or Latino	5 (10.6)	8 (14.3)	12 (22.2)	20 (18.2)	25 (15.9)
Not Hispanic or Latino	40 (85.1)	47 (83.9)	42 (77.8)	89 (80.9)	129 (82.2)
Not reported	2 (4.3)	0	0	0	2 (1.3)
Unknown	0	1 (1.8)	0	1 (0.9)	1 (0.6)
Race, n (%)					
American Indian or Alaska Native	3 (6.4)	3 (5.4)	2 (3.7)	5 (4.5)	8 (5.1)
White	41 (87.2)	52 (92.9)	49 (90.7)	101 (91.8)	142 (90.4)
Other	3 (6.4)	1 (1.8)	3 (5.6)	4 (3.6)	7 (4.5)
BMI (kg/m^2)					
Median (min, max)	22.43 (13.2, 41.0)	19.75 (13.8, 56.9)	22.51 (14.5, 35.5)	21.08 (13.8, 56.9)	21.19 (13.2, 56.9)
Screening Phe concentration (µmol/L)[a]					
n	21	26	26	52	73
Mean (SD)	629.10 (183.582)	649.38 (219.687)	592.46 (185.039)	620.92 (203.145)	623.27 (196.487)
Median	559.00	594.65	583.35	584.50	582.30
Min, max	405.5, 1088.5	409.0, 1333.6	310.3, 955.1	310.3, 1333.6	310.3, 1333.6
PKU first diagnosed at birth, n (%)					
Yes	36 (76.6)	38 (67.9)	34 (63.0)	72 (65.5)	108 (68.8)
No	11 (23.4)	18 (32.1)	20 (37.0)	38 (34.5)	49 (31.2)
Classical PKU subjects, n (%)					

Yes	17 (36.2)	8 (14.3)	11 (20.4)	19 (17.3)	36 (22.9)
No	30 (63.8)	48 (85.7)	43 (79.6)	91 (82.7)	121 (77.1)
Receiving pegvaliase or BH_4 therapy at screening					
No	43 (91.5)	40 (71.4)	46 (85.2)	86 (78.2)	129 (82.2)
Yes	4 (8.5)	16 (28.6)	8 (14.8)	24 (21.8)	28 (17.8)
pegvaliase	0	0	1 (1.9)	1 (0.9)	1 (0.6)
BH_4	4 (8.5)	16 (28.6)	7 (13.0)	23 (20.9)	27 (17.2)
Both	0	0	0	0	0
Documented as BH_4-responsive					
No	0	0	0	0	0
Yes	1 (2.1)	21 (37.5)	10 (18.5)	31 (28.2)	32 (20.4)
Missing	46 (97.9)	35 (62.5)	44 (81.5)	79 (71.8)	125 (79.6)

Abbreviations: BH_4, tetrahydrobiopterin; BMI, body mass index; CSR, clinical study report; FAS, Full Analysis Set; max, maximum; min, minimum; Phe, phenylalanine; PKU, phenylketonuria; SD, standard deviation
[a] Average of the most recent Phe concentration inclusive of the screening value.

Demographics and baseline characteristics for FAS are further summarized by Phe reduction randomization strata (≥15%-<30% or ≥30%). In general, the demographics and baseline characteristics for the FAS were similar to those for the Safety Analysis Set.

Disease characteristics for participants who did not progress beyond Part 1 were generally similar to those of participants in Part 2 both for SAS and FAS.

- **Numbers analysed**

A total of 156 of the 157 enrolled patients were assessed for Responsiveness to Sepiapterin in Part 1 of the study. 1 patient (who had classical PKU) withdrew consent on Day 2 of Part 1.

110 patients were randomized in Part 2, to receive either sepiapterin (n=56) or placebo (n=54) treatment being the **Full Analysis Set**. Of those, 98 patients had a Phe Reduction from Baseline ≥30% during Part 1 and were the **primary analysis population**. They were randomized to receive Sepiapterin or Placebo (49/49) in part 2 of the study.

- **Outcomes and estimation**

In **Part 1** of the study, after 14 days of sepiapterin treatment, 66.0% (103/156) of participants demonstrated a ≥30% reduction in blood Phe in response to sepiapterin, with a mean reduction of 462.2 µmol/L (65% reduction from baseline). Further, 114 (73.1%) of 156 participants demonstrated a ≥15% reduction in blood Phe level in response to sepiapterin.

Positive results were also demonstrated in the 36 cPKU patients (45.7% (16/35) of participants demonstrated a ≥30% reduction from baseline blood Phe concentration with a mean reduction of 582.5 µmol/L (60% reduction from baseline), participants that were documented as nonresponsive to BH_4 therapy [(n=56), 42.9% (24/56) responded to sepiapterin (≥30% reduction in blood Phe levels in baseline Phe, with a mean reduction in blood Phe concentration of 404.3 µmol/L (55% reduction from baseline)] and patients receiving BH_4 therapy at the time of screening [27 participants, 85.2% (23/27) responded to sepiapterin (≥30% reduction in blood Phe levels in baseline Phe), achieving a 48% reduction from the Phe concentration achieved on sapropterin therapy].

Part 2 results demonstrated a significant and important impact of sepiapterin treatment on blood Phe concentration at 6 weeks compared to baseline in the majority of subjects treated.

Considering the **primary analysis population** 89.8% (44/49) of the participants in the sepiapterin group achieved a reduction of ≥30% during Part 2 compared to 10.2% (5/49) in the placebo group. By Week 6, mean blood Phe concentration decreased significantly (decrease of 410.1 µmol/L, 63%), whereas Phe concentration remained relatively unchanged with placebo (decrease of 16.2 µmol/L, +1.4%). In the **FAS**, a statistically significant ($p<0.0001$) difference in the mean change in blood Phe levels from baseline to Weeks 5 and 6 was observed in the sepiapterin arm following treatment with sepiapterin compared with placebo. By Week 6, mean blood Phe levels decreased significantly in the sepiapterin arm (LS mean change of -289.59 µmol/L), whereas Phe levels remained relatively unchanged in the placebo arm (LS mean change of 65.31 µmol/L).

Results were more impressive in the previously determined responder sub-population but nevertheless were seen in FAS. There were no influence of sex and age on treatment effects.

Approximately 90% of subjects achieved their age-appropriate European target blood Phe concentration, and 22% of subjects achieved Phe concentration that are considered normalized.

cPKU patients, known to have a less impressive response to treatments also showed an overall very good response in terms of Phe serum levels after the 6 weeks treatment (n=19; 63% lowering of blood Phe concentrations versus baseline); of the 15 with cPKU that had a Phe reduction in part 1 of >30%, significant ($p<0.0001$) larger reduction in mean blood Phe was seen in subjects with cPKU on sepiapterin *vs* placebo (-523.46 µmol/L vs -42.13) µmol/L), representing a 69% decrease from baseline.

The study was not sufficiently powered to detect differences based on the subpopulations, the sample size was limited for each subgroup comparison and type I error was not controlled for these evaluations which are not considered in the multiplicity adjustment procedure. Consequently, reference to these descriptive results in the SmPC was updated with no reference to the p-value.

The LS mean (SE) difference in mean change from baseline *versus* placebo increased significantly over time although significant results were achieved at all assessed time points (1-2; 3-4 weeks, 5-6 weeks).

The proportion of **participants with baseline Phe ≥600 µmol/L, ≥360 µmol/L and Phe ≥120 µmol/L who achieved Phe levels below the defined threshold** in Part 2 were consistently significantly greater ($p<0.0001$) following treatment with sepiapterin compared with placebo.

Treatment efficacy was also demonstrated in subjects who were documented as **nonresponsive to BH_4**.

Patients there were treated with sapropterin at study screening, were also randomized after part 1 of the study. The improvement was even more significative with a longer treatment period, with patients on placebo returning to pre-study Phe levels. A significant ($p<0.0001$) difference in the mean change in blood Phe concentration from baseline to Weeks 5 and 6 was demonstrated with sepiapterin (n=14) compared with placebo (n=7). At Week 6, in subjects who received sepiapterin, mean blood Phe concentration decreased significantly (decrease of 438.28 µmol/L, 63%).

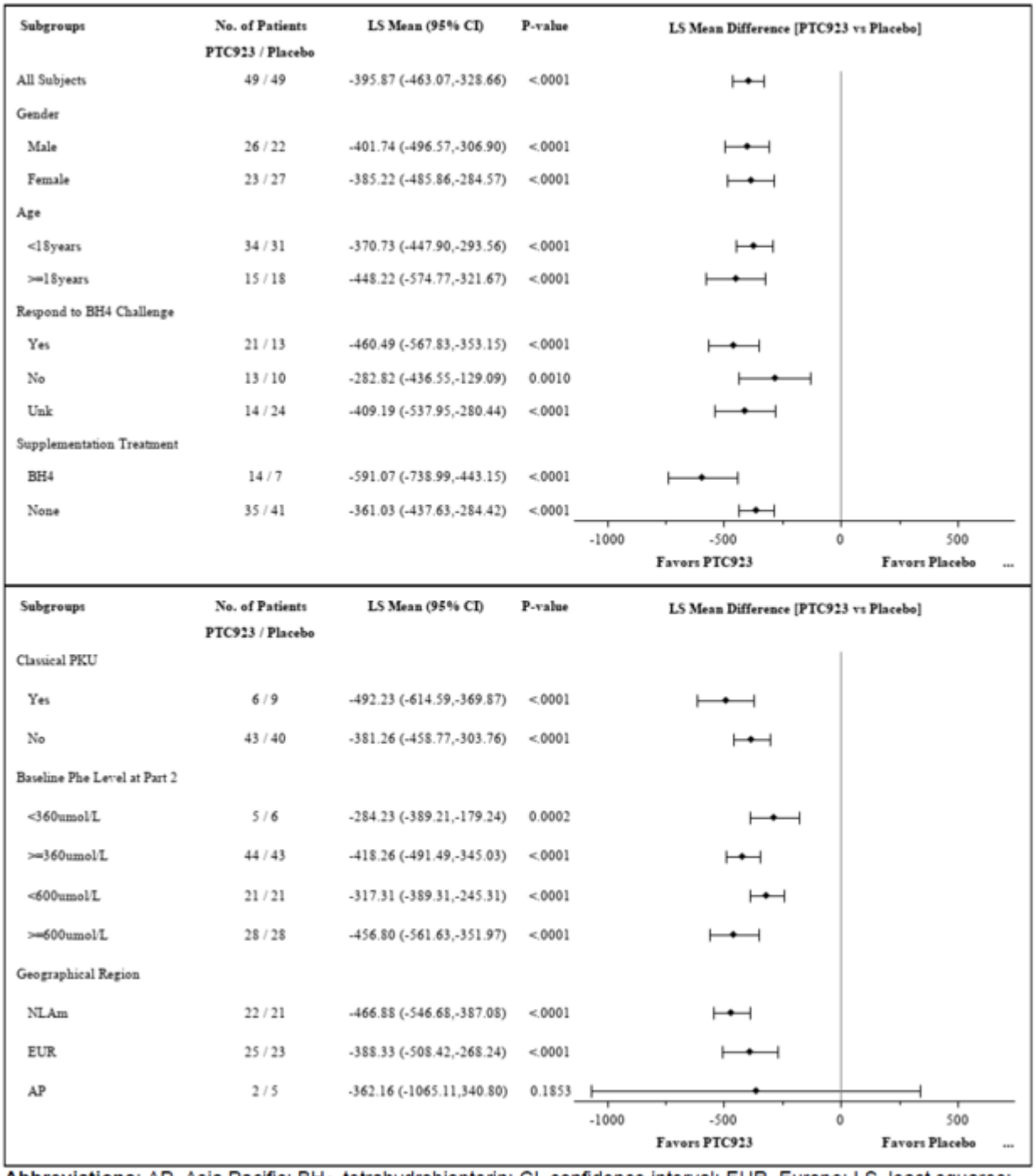
A **decrease in Phe:Tyr ratio** has been demonstrated following a decrease in Phe and no changes in Tyr levels. This decreased Phe:Tyr is associated with improved neuropsychological outcome.

- **Ancillary analyses**

Ancillary analyses

Results of the subgroup analyses of the primary efficacy endpoint (gender, age, response to BH_4 challenge, participants who enter the study and were receiving supplementation treatment, biochemically diagnosed classical PKU, baseline Phe level of Part 2, change of diet during Part 2, and geographic region) are generally consistent with the results of the primary/secondary efficacy analysis for both the primary analysis population and FAS.

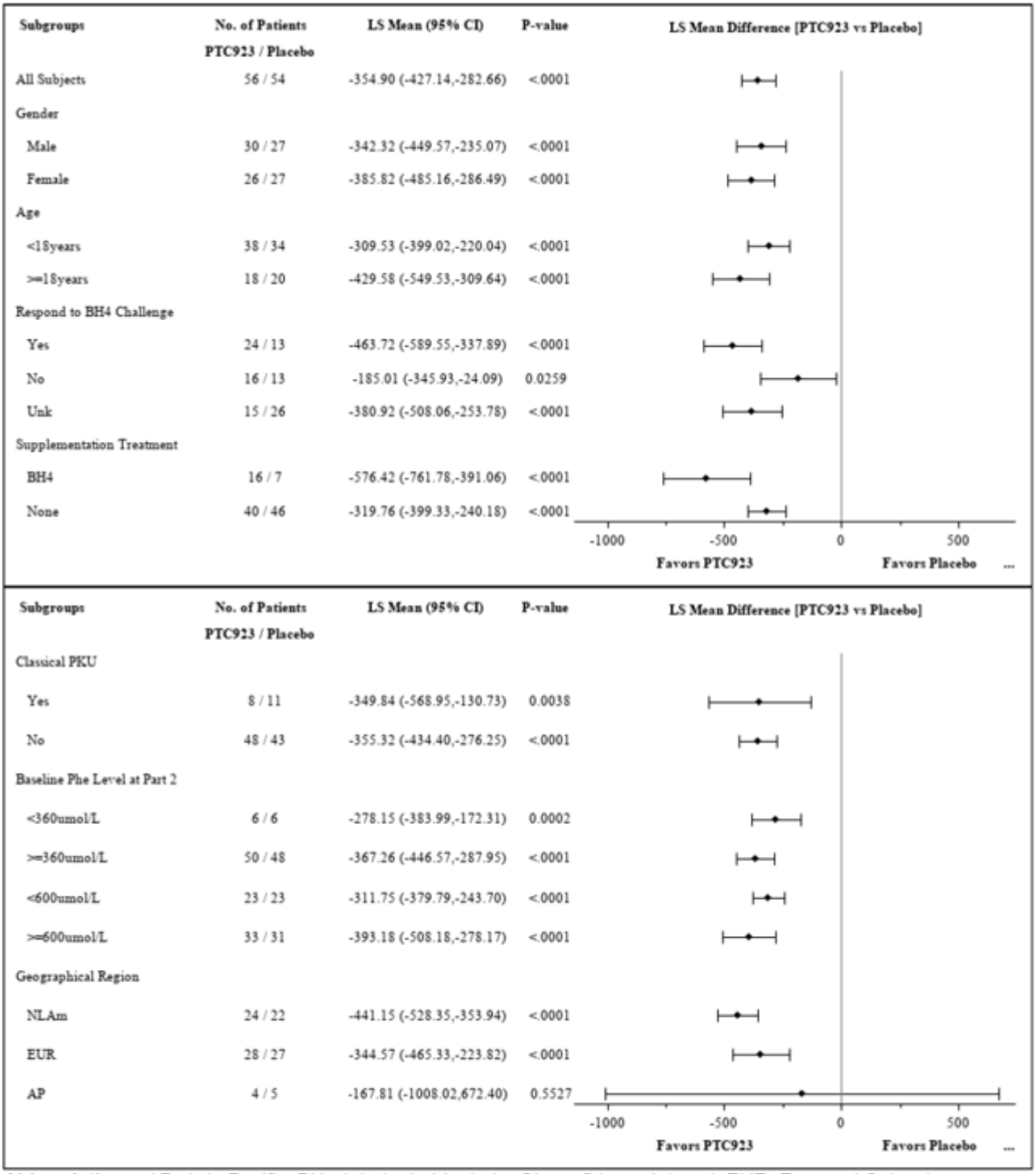
Figure 11: **Forest Plot for LS mean difference in blood Phe levels from baseline to Weeks 5 and 6 in Part 2 by subgroup (Full Analysis Set with Phe reduction from baseline ⩾30% during Part 1)**



Abbreviations: AP, Asia Pacific; BH_4, tetrahydrobiopterin; CI, confidence interval; EUR, Europe; LS, least squares; MMRM, mixed model of repeated measures; NLAm, North and Latin America; Phe, phenylalanine; PKU, phenylketonuria; PTC923, sepiapterin; Unk, unknown

Note: For a subgroup with nonevaluable result in the MMRM analysis due to very limited participants, it is not shown on the plot.

Figure 12: **Forest Plot for LS mean difference in blood Phe levels from baseline to Weeks 5 and 6 in Part 2 by subgroup (Full Analysis Set)**



Abbreviations: AP, Asia Pacific; BH_4, tetrahydrobiopterin; CI, confidence interval; EUR, Europe; LS, least squares; MMRM, mixed model of repeated measures; NLAm, North and Latin America; Phe, phenylalanine; PKU, phenylketonuria; PTC923, sepiapterin; Unk, unknown

Note: For a subgroup with nonevaluable result in the MMRM analysis due to very limited participants, it is not shown on the plot.

- **Summary of main efficacy results**

The following tables summarise the efficacy results from the main studies supporting the present application. These summaries should be read in conjunction with the discussion on clinical efficacy as well as the benefit risk assessment (see later sections).

Table 15. Summary of Efficacy for Study PTC923-MD-003-PKU

Title: A Phase 3 Study of PTC923 in Subjects with Phenylketonuria		
Study identifier	PTC923-MD-003-PKU Clinicaltrials.gov: NCT05099640 EudraCT number: 2021-000474-29	
Design	This was a Phase 3, global, double-blind, placebo-controlled, randomized, multi center efficacy study of sepiapterin versus placebo in participants with PKU of all ages.	
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:	42 days (randomized treatment Phase [Part 2]) 28-35 days sepiapterin responsiveness assessment (Part 1) Open label extension (PTC-MD-004-PKU)
Hypothesis	Superiority of sepiapterin versus placebo	
Treatments groups	Group A – sepiapterin	Part 1: Open label sepiapterin (7.5 mg/kg for participants 0 to <6 months of age, 15 mg/kg for participants 6 to <12 months of age, 30 mg/kg for participants 12 months to <2 years of age, and 60 mg/kg for participants ≥2 years of age) for 14 days starting on Day 1. 157 subjects enrolled and treated with sepiapterin in Part 1 Part 2: total 110 subjects randomized in Part 2, 56 subjects randomized to sepiapterin 20 mg/kg daily for Days 1 to 14 (i.e., Weeks 1 and 2), then sepiapterin 40 mg/kg daily for Days 15 to 28 (i.e., Weeks 3 and 4), then sepiapterin 60 mg/kg daily for Days 29 to 42 (i.e., Weeks 5 and 6).

	Group B – placebo	Part 2: total 110 subjects randomized in Part 2, 54 subjects randomized to placebo to match the 20 to 40 to 60 mg/kg dose escalation of those randomized to sepiapterin
Endpoints and definitions	Primary endpoint	Mean change in blood Phe levels from baseline to Weeks 5 and 6 (average over a 2-week period) in the Part 2 double-blind phase
	Secondary endpoints	1. Proportion of subjects with baseline Phe levels ≥600 µmol/L who achieve Phe levels <600 µmol/L at the end of the double-blind treatment period 2. Proportion of subjects with baseline Phe levels ≥360 µmol/L who achieve Phe levels <360 µmol/L at the end of the double-blind treatment period 3. Mean change and percent change from baseline in blood Phe levels at each dose level 4. PK concentrations and parameters of sepiapterin and BH_4 5. Severity and number of TEAEs, clinical laboratory tests, vital signs, physical examinations, and ECGs
Database lock	11 May 2023	
Results and Analysis		

Analysis description	**Primary Analysis**		
Analysis population and time point description	The primary analysis population was defined for the stratum of participants with mean percent reduction in blood Phe levels of ≥30% during Part 1. The primary endpoint was the mean change in blood Phe levels from baseline to Weeks 5 and 6 (average over a 2-week period) in the Part 2 double-blind phase		
	Treatment group	Full analysis set (subjects with Phe reduction from baseline of ≥30% during Part 1)	
Descriptive statistics and estimate variability		Sepiapterin	Placebo
	Number of subjects (FAS with ≥30% Phe reduction vs baseline in Part 1)	49	49
	Actual change from baseline in blood Phe concentration (mean)	-410.07	-16.19
	Standard deviation	204.442	198.642
Effect estimate per comparison	LS Mean estimate of mean change from baseline (FAS with ≥30% Phe reduction vs baseline in Part 1)	Sepiapterin vs placebo	sepiapterin vs placebo
		Difference between sepiapterin and placebo	-395.87
		SE 95% CI	33.848 (-463.07, -328.66)
		p value	<0.0001
	Secondary analysis based on Full analysis set	Full analysis set	
		Sepiapterin vs placebo	sepiapterin vs placebo
		Difference between sepiapterin and placebo	-354.90
		SE 95% CI	36.435 (-427.14, -282.66)
		p value	<0.0001
Analysis description	**Secondary analysis**		
	Treatment group	Full Analysis Set with Phe Reduction from Baseline ≥30% During Part 1)	
Descriptive statistics and estimate variability		Group A	Group B
	Proportion of Participants With Baseline Phe Levels ≥600 µmol/L Who Achieved Phe Levels <600 µmol/L in Part 2	(n=28)	(n=30)
	Achieved	26 (92.9%)	9 (30.0%)

	Not Achieved	2 (7.1%)	21 (70.0%)
Effect estimate per comparison	Odds ratio (Group A vs Group B)	Group A vs Group B	Group A (sepiapterin) vs Group B (placebo)
		Odds ratio sepiapterin vs placebo	30.33
		95% CI	(5.3, 294.24)
		p value	<0.0001
	Proportion of Participants With Baseline Phe Levels ≥360 µmol/L Who Achieved Phe Levels <360 µmol/L in Part 2 (Full Analysis Set With Phe Reduction From Baseline ≥30% During Part 1)	Full Analysis Set with Phe Reduction from Baseline ≥30% During Part 1)	
		Group A	Group B
		(n=44)	(n=43)
	Achieved	37 (84.1%)	4 (9.3%)
	Not achieved	7 (15.9%)	39 (90.7%)
	Odds ratio (Group A vs Group B)	Full Analysis Set with Phe Reduction from Baseline ≥30% During Part 1)	
		Group A vs Group B	Group A (sepiapterin) vs Group B (placebo)
		Odds ratio sepiapterin vs placebo	51.54
		95% CI	(12.28, 245.34)
		p value	<0.0001

Abbreviations: D, day; ECG, electrocardiogram; LS, least squares; M, month; Phe, phenylalanine; PKU, phenylketonuria; QOL, quality of life; SD, standard deviation; TEAE, treatment emergent adverse event

A broad indication covering adults and paediatric patients regardless of age was initially not supported. The low number of exposed patients between the age of 1-2 years of age in the part 1 of this study and the open-label long-term study and the absence of patients under the age of one was of concern. The Applicant was requested to discuss whether efficacy and safety data could be extrapolated to patients of this age category (0-2 years of age), or, alternatively, restrict the age limit of the indication. The Applicant provided updated data (data cut-off date 02 September 2024) from the ongoing study PTC923-MD-004-PKU evaluating the long-term safety of sepiapterin as well as the impact of sepiapterin treatment in changes in dietary Phe/protein consumption. This new data is discussed further below in study PTC923-MD-004-PKU section. Nevertheless, in summary, the data on Dietary Phe Tolerance Assessment (primary efficacy endpoint) was only available for 4 participants aged <2years of age. Following a new request for updated data, the Applicant updated the number of patients < 2 years of age to 33 (previously 15), 8 aged <1 year. Of the 33 patients, 8 completed the 26 weeks Dietary Phe Tolerance Assessment, with 7 other still ongoing.

The study population in the pivotal study consisted of a stratum of patients who experienced a ≥ 15% reduction of blood Phe levels after receiving 60 mg/kg/d of sepiapterin for 14 days. The Applicant was asked to reflect this in section 4.2 of the SmPC. During the procedure, the Applicant argued that data exists supporting the Phe blood level improvement beyond the 14 days timeframe in some patients. However, as described by the Applicant, the data supporting this is limited, under individual patients, and not controlled. Numbers are quite residual and cannot be considered of the same "quality" level as the ones generated in the double-blind part of the study. Section 4.2 of the SmPC was updated as followed to comply with this:

"No controlled efficacy and safety data are available in patients who do not experience a reduction of 15% or greater reduction in blood Phe levels after receiving sepiapterin for 14 days".

Patients documented as 'BH_4 nonresponsive' prior to study entry had received appropriate standard of care to assess their BH_4 responsiveness per local guidelines in conjunction with appropriate dietary restriction. In general, a reduction of 30% or greater in blood Phe was used as the criterion for sapropterin response. No information is available regarding diet compliance at the time of the BH_4 responsiveness testing period. But the Applicant states that the Kuvan SmPC states that "existing dietary protein and Phe intake should not be modified during the evaluation (responsiveness) testing."

The dataset used to assess the % of subjects that achieved their age-appropriate European target blood Phe concentration is of 67 patients (of the total 110 patients). Considering the FAS, 75 of the total 110 participants had blood Phe concentration above target at Part 2 study initiation. Results for the FAS were similar to the PAP, with approximately 80% to 85% achieving blood Phe reductions that reached age-appropriate European target levels.

The LS mean (SE) difference in mean change from baseline versus placebo increased significantly over time although significant results were achieved at all assessed time points (1-2; 3-4 weeks, 5-6 weeks). Improvement in Phe levels with increasing treatment time triggered a discussion on the time for treatment responder/non-responder classification, that in the part 1 of this study was based on the response at week 2 of treatment. The Applicant reiterates that responsiveness testing in Part 1 of the study demonstrated that 14 days is an adequate time to determine a participant's response to sepiapterin.

2.6.5.3. Clinical studies in special populations

Studies were not conducted in special populations.

2.6.5.4. In vitro biomarker test for patient selection for efficacy

Not applicable.

2.6.5.5. Analysis performed across trials (pooled analyses and meta-analysis)

Not applicable.

2.6.5.6. Supportive study(ies)

Study PTC923-MD-004-PKU is an ongoing, Phase 3, multicenter, open-label study evaluating the long-term safety of sepiapterin as well as the impact of sepiapterin treatment in changes from baseline in dietary Phe/protein consumption.

Although the study will include *de novo* patients and patients that rolled from study PTC923-MD-003-PKU.

The primary objectives were long-term safety of sepiapterin and changes from baseline in dietary Phe/protein consumption (Dietary Phe tolerance) in patients with blood Phe level <360 µmol/L. Dietary Phe tolerance aimed to evaluate the amount of phenylalanine supplement tolerated (i.e., phenylalanine dietary consumption while maintaining blood phenylalanine level <360 µmol/L) from baseline to week 26.

Secondary objectives included QOL using the PKU-QOL scale, and the EQ-5D score based on age groups.

As for previous studies, both patients naïve to other than restrict diet treatment and patients treated with sapropterin or pegvaliase will be enrolled up to 200 subjects.

For all study subjects, the treatment phase consists of open-label (OL) treatment with sepiapterin administered orally once a day for a minimum of 12 months or until they experienced lack of efficacy or adverse events (AEs) that led to discontinuation, withdrawal from treatment, or until sepiapterin is authorized and commercially available. Proposed doses are consistent to the ones used in previous trial(s).

After the first month of OL treatment Phe levels will be used to decide if the patients can enter the Dietary Phe Tolerance Assessment (should have Phe (<360 µmol/L).

From the methodology it seems that although an average of 3 measurements in a 10-day period is requested, it might well end up that decision could be made with 2 or even on one Phe determination only. The same strategy is to be implemented for decisions on dietary protein increase every 2 weeks during the Dietary Phe Tolerance Assessment. The impact of this strategy on the consistency of patients and on final results was requested to be discussed and commented. The Applicant confirmed that indeed it would be allowable to have less than 3 blood Phe measurements to determine whether a participant would qualify for the Dietary Phe Tolerance Assessment or not. As per current date, only 4 out of 169 participants had less than 3 measurements contributing to their mean blood Phe calculation in Month 1 of the study (all had 2 measurements). The Applicant considers that the inclusion of at least 1 or 2 values for each 2-week mean would provide sufficient representation of the average blood Phe levels during the assessment timeframe.

The average Blood Phe level was of 360 µmol/L by week 26. Monitored increases of Phe blood levels up to the maximum target were allowed to test the maximum individually tolerated Phe intake for each participant while maintaining the target blood Phe concentration (<360 µmol/L; excellent control for ages >12 years, and the target range for patients <12 years per EU PKU treatment guidelines).

For those participating in the Dietary Phe Tolerance Assessment, every 2 weeks Phe dose increments are pre-defined according to previous Phe blood levels, and patients will have Phe increments up to the maximum protein intake tolerated.

Within the first interim analysis provided by the Applicant, the primary efficacy assessment was carried out on the Dietary Phe Tolerance Analysis Set, which comprised of 73 subjects with mean blood Phe <360 µmol/L at M1D5, M1D10, and M1D14.

Results of the subgroup analysis of the change from baseline to Week 26 in dietary Phe consumption (gender, age category, classical PKU diagnosis, and screening Phe level, response to BH_4 challenge, supplementation therapy) are generally consistent with the results of the primary analysis.

57.1% (12/21) and 44.0% (11/25) of subjects reached their age-adjusted RDA and 1.5-fold this limit (respectively) by Week 16; 36.0% (9/25) of subjects reached 2-fold their age-adjusted RDA by Week 20 and all maintained this through the 26-week dietary Phe tolerance assessment.

In the overall population of subjects from Study PTC923-MD-003-PKU and the set of subjects who received sepiapterin in Part 2 of Study PTC923-MD-003-PKU, blood Phe remained generally unchanged throughout Study PTC923-MD-004-PKU as of the data cut-off date. Subjects who received placebo in Part 2 of Study PTC923-MD-003-PKU had a marked decrease in blood Phe levels after transition into Study PTC923-MD-004-PKU but according to data provided, in average, they had Phe blood levels consistently above the target threshold between month 6 and 16.

Overall and in the Dietary Phe Tolerance Analysist Set, blood Tyr and median Phe:Tyr ratio remained generally constant through the end of the study. Some variations in later months occurred and will be further clarified upon full data analysis.

Blood Phe levels remained generally stable during the study in the Dietary Phe Tolerance Analysis Set and in the overall population but increased in the subjects with blood Phe ≥360 µmol/L at screening. However, results are difficult to interpret due to the limited number of subjects who had completed the later time points at the time of data cut-off.

As of the data cut-off date (22 September 2023), a total of 104 subjects had been treated with sepiapterin.

The Applicant provided, as requested, an updated data (data cut-off date 02 September 2024) from the ongoing study PTC923-MD-004-PKU evaluating the long-term safety of sepiapterin as well as the impact of sepiapterin treatment in changes in dietary Phe/protein consumption. This included data on 65 additional participants, for a total of 169 participants (129 participants completed 6 months of treatment, while 95 participants completed 12 months of treatment).

Table 16. Summary of Efficacy for Study PTC923-MD-004-PKU

Title: A Phase 3, Open Label Study of PTC923 (sepiapterin) in Phenylketonuria		
Study identifier	PTC923-MD-004-PKU Clinicaltrials.gov: NCT05166161 EudraCT number: 2021-000497-28	
Design	Study PTC923-MD-004-PKU is an ongoing, Phase 3, multicenter, open-label study in subjects with PKU. Eligible subjects are: Feeder subjects: those who have completed a Phase 3 PTC-sponsored feeder study (including Study PTC923-MD-003-PKU)	
	Duration of main phase:	A minimum of 12 months
Hypothesis	For the primary efficacy endpoint, the hypothesis testing will be made at the end of the study. The purpose of the interim analysis was to provide data available in a descriptive way to support regulatory submission needs.	
Treatments groups	All subjects receive sepiapterin Powder for Oral Use, which is packaged in sachets of 250 mg and 1000 mg. Upon study entry, subjects are assigned a weight-based dose according to their age, as follows: • 0 to <6 months of age: up to 7.5 mg/kg/day • 6 to <12 months of age: up to 15 mg/kg/day	

	• 12 months to <2 years of age: up to 30 mg/kg/day • ≥2 years of age: up to 60 mg/kg/day	
	Group A	Feeder subjects with blood Phe ≥360 µmol/L
	Group B	Feeder subjects with blood Phe <360 µmol/L
Endpoints and definitions	Primary safety endpoint	Severity and number of TEAE, clinical laboratory tests, vital signs, and physical examinations over the treatment period
	Primary efficacy endpoint	Mean change in Phe consumption from baseline to Week 26
	Secondary endpoints	1. Change from baseline in PKU-QOL score by age group 2. Change from baseline in EQ-5D index score by age group 3. PK assessment of sepiapterin and BH_4 concentrations in plasma
Database lock	Interim data cut date: 02 September 2024	

Results and Analysis

Analysis description	**Primary Analysis**
Analysis population and time point description	The primary efficacy endpoint was the change from baseline in dietary Phe/protein consumption. Descriptive statistics were used to summarize the daily dietary Phe consumption (mg/kg/day) data and the prescribed daily dietary Phe (mg/kg/day), and the changes in daily dietary Phe consumption (mg/kg/day) from baseline were fitted by a mixed model repeated measures (MMRM) model, for all subjects and certain subgroups based on the Dietary Phe Tolerance Analysis Set. Dietary Phe tolerance was assessed from M2D1. Adjustments were made in dietary Phe consumption, as appropriate, every 2 weeks. The study included the following analysis sets: • Dietary Phe Tolerance Analysis Set: Subjects whose mean blood Phe at M1D5, M1D10, and M1D14 was <360 µmol/L and who receive at least 1 dose of study drug during the Dietary Phe Tolerance Assessment period. • Safety Analysis Set: All subjects who received at least 1 dose of study drug. • Full Analysis Set: All subjects who received at least 1 dose of study drug and conducted at least 1 postbaseline efficacy measurement, except for non-responsive non-feeder study subjects. • PK Analysis Set: All subjects who had at least 1 measurable plasma concentration of sepiapterin or BH_4.

		Change in Daily Dietary Phe Consumption from Baseline to Week 26 (dietary Phe tolerance assessment set)	
Descriptive statistics and estimate variability	Treatment group	Baseline	Week 26
	Number of subjects	102	81
	Dietary Phe consumption (mg/kg/day) (mean)	27.564	62.521
	Standard deviation	18.0096	41.5230
Effect estimate per comparison	LS Mean estimate of mean change from baseline	Comparison groups Baseline vs Week 26	
		Mean change versus baseline	36.466
		SD	36.8648

Abbreviations: D, day; ECG, electrocardiogram; LS, least squares; M, month; Phe, phenylalanine; PKU, phenylketonuria; QOL, quality of life; SD, standard deviation; TEAE, treatment emergent adverse event

Daily use of sepiapterin permitted an approximately 2.3-fold increase in mean daily Phe consumption (27.6 mg/kg/day at baseline versus 62.5 mg/kg/day at Week 26). The vast majority of participants achieved significant increases in dietary protein/Phe intake over the course of the Dietary Phe Tolerance Assessment, while mean blood Phe did not exceed the target of 360 µmol/L by Week 26.

Similarly, to study PTC923MD-003-PKU responsiveness to sepiapterin definition (achievement of a ≥15% reduction in blood Phe) the responder rate from Part 1 of Study PTC923-MD-004-PKU was 73.1%. Section 5.1 of the SmPC was updated with this information. This included 14 patients <2 years of age (5 < 1 year and 9 aged 1 to < 2 years of age). Nine (9) patients had a dietary Phe assessment (3 < 1year of age). Among those participants, 12 participants were treated only in Study PTC923-MD-004-PKU and 2 were enrolled in Study PTC923-MD-004-PKU after completing the pivotal study. Of note 1 patient below 2 years old included in the part 1 of the PTC923-MD-003-PKU study did not respond and thus did not participate to PTC923-MD-004-PKU.

Among the 12 patients who were only treated in study PTC923-MD-004-PKU, 9 (66.7 %) achieved a ≥15% reduction in blood Phe level during the first 2 weeks of treatment and were considered responders. The percentage of responders is comparable with the one observed in patients aged > 2 years old (73.9%).

All 9 patients who were eligible to continue the study underwent the Dietary Phe Tolerance Assessment (primary efficacy endpoint). Results at Week 26 indicate an increase in dietary Phe consumption *versus* baseline while maintaining a blood Phe level < 360 µg/L in line with what was observed in patients aged > 2 years old (mean (SD) change from baseline at week 26 63.653 (36.6697) and 35.054 (36.354.88) respectively).

Following a new request for updated data (between D120 and D195; cut-off 13 February 2025), the Applicant has submitted data from 33 participants <2 years of age, 8 of which are < 1 year of age.

The overall mean duration of exposure is 165.1 days, with those participating in the Dietary Phe Tolerance Assessment having mean exposure of 284.1 days.

The data demonstrate that response to sepiapterin (defined as ≥15% reduction in blood Phe) is similar across all age groups. A consistent response to sepiapterin between 62.5% and 73.2% was seen over 2 weeks of therapy regardless of age.

The final CSR for this study will be submitted once available via a Type-II variation post-authorisation.

Table 17. Response Rate to Sepiapterin Treatment by Age Group (Overall in Study PTC923-MD-003-PKU and Study PTC923-MD-004-PKU)

Category, n/N (%)	Age Group			
	≥2 Years n (%)	<2 Years n (%)	≥1 to 2 Years	<1 Year
Participants dosed	N=154	N=33	N=25	N=8
Participants who achieved a ≥15% reduction in blood Phe in the first 2 weeks of treatment	112 (73.2)[a]	22 (66.7)	17 (68.0)	5 (62.5)
Participants who achieved a ≥30% reduction in blood Phe in the first 2 weeks of treatment	101 (66.0)[a]	18 (54.5)	13 (52.0)	5 (62.5)
Percent of participants achieving <360 µmol/L at Week 2	N=44	N=23[b]	N=18[b]	N=5[b]
	37/44 (84.1)	17 (74.0)	13 (72.0)	4 (80.0)

Abbreviations: Phe, phenylalanine
[a]One participant did not have Week 1 and/or Week 2 value, so denominator is N=153.
[b]Values from participants in the Dietary Phe Tolerance Assessment.
Cut-off date 13 February 2025.

In addition, results were provided from 8 participants who are <2 years of age and who completed the 26 weeks of the Dietary Phe Tolerance Assessment, and findings from 7 additional participants who are currently in the ongoing Dietary Phe Tolerance Assessment. Moreover, long-term data of blood Phe for ≥24 months are also available. All 8 participants who have completed the Dietary Phe Tolerance Assessment exceeded the recommended daily allowance (RDA) by Week 10 and continued to increase their protein intake over the remaining 16 weeks. At Week 26, a 2.9-fold increase in mean daily Phe consumption was observed in participants <2 years of age, which is similar to that of 2.3-fold increase for participants ≥2 years of age. The percentage of participants <2 years of age who maintained a 2-week mean blood Phe to Week 26 of <360 µmol/L was 86.6%. which is consistent with the results in older patients (75.1%). Similar results are seen for the interim results of the 7 participants who have not yet completed the 26-week Dietary Phe Tolerance Assessment for both the increased Phe tolerance and maintenance of blood Phe of <360 µmol/L. Stratification of the data by <1 and ≥1 to <2 years of age shows sepiapterin is similarly effective in increasing Phe tolerance in both age groups.

Sepiapterin maintained blood Phe below or in close proximity to 360 µmol/L for ≥24 months in participants <2 year of age.

The newly submitted data demonstrates that

- Sepiapterin provides strong and consistent efficacy in both blood phenylalanine (Phe) lowering and dietary Phe tolerance in participants that are <2 years of age that is comparable to participants ≥2 years of age.

  Sepiapterin demonstrates consistent efficacy across the <1-year and ≥1- to <2-year age groups.

- Sepiapterin in the <2-year-old population has a high response rate (~67%) and results in a large proportion of participants (~87%) maintaining 2-week mean blood Phe control over 26 weeks within the European guideline-recommended <360 µmol/L.

Both in the overall population and in the Dietary Phe Tolerance Analysist Set, blood Tyr concentration and the median Phe:Tyr ratio remained generally unchanged through Month 34.

Preliminary results for change from baseline for 2 composite scores of the Phenylketonuria Quality of Life (PKU-QOL) scale, Overall Impact of PKU and Overall Impact of Dietary Protein, show a clear trend for the efficacy of sepiapterin in improving key facets of HRQoL for participants with PKU. Following a request during the procedure, the Applicant has elaborated on the QoL results. Although still preliminary, the data suggest that sepiapterin treatment is associated with meaningful, clinically relevant improvement in the QOL of PKU patients.

2.6.6. Discussion on clinical efficacy

Sepiapterin is being studied for the treatment of hyperphenylalaninaemia in patients with PKU.

The clinical efficacy of sepiapterin was assessed in three clinical studies:

- A completed, pivotal, Phase 3, placebo-controlled efficacy and safety study (PTC923MD-003-PKU)
- An ongoing, Phase 3, long-term, open label, efficacy and safety study (PTC923MD004PKU)
- A completed, Phase 2, head-to-head comparator study (PKU-002)

The studies were developed:

- to propose the dosing regimen with the best safety and efficacy profile for sepiapterin (PKU-002)
- to compare 2 dose levels - 20 vs 60mg/kg/day (PKU-002)
- to confirm the efficacy of sepiapterin in patients with different PKU severity including patients with classic PKU (PKU-002 and PTC923MD-003-PKU)
- to compare the response between sepiapterin and sapropterin and demonstrate additional clinical benefit of sepiapterin treatment in patients who respond insufficiently or who are not responsive to sapropterin (PKU-002, PTC923MD-003-PKU and PKU-002 and PTC923MD-004-PKU)
- assess de impact of sepiapterin on liberalisation of diet restrictions (PTC923MD-004-PKU)

PTC923MD-003-PKU was a multicenter, placebo-controlled, double-blind, randomized study where patients after having been classified as responders to sepiapterin in an initial part 1 (minimum of decrease of 15% of Phe blood levels after 14 days of treatment) were enrolled in a 6-week placebo control trial. Use of placebo is acceptable in light of the available alternatives and is justified as patients were still on a restricted Phe diet (current SOC). Excluding non-responders from part 2 (placebo-controlled) prevented patients' exposure for 6 weeks to a drug to which they did not respond in part 1. As a patient-based response is to be the standard current practice, this enrich responder sample procedure is considered acceptable. All patients were instructed and monitored to use the same diet throughout the study to control the effect of diet on the response to the treatment implemented.

The study population aims to reflect the intended indication and the study inclusion criteria were developed to include the broadest possible criteria to reflect the wider PKU population (paediatric/adult; different

severity/metabolic control status and subjects with cPKU although capped at 20% in this study). Balanced randomization of patients with different Phe serum levels (different severity/resistance do diet) was important to better assess the overall efficacy of the treatment. Also, having included a subset of population treated with sapropterin at the time of screening allowed for assessment of comparative response (following preliminary results from study PKU-002). Only one patient had previously been treated with pegvaliase not allowing to retrieve any clinically relevant conclusion. The baseline characteristics are rather similar between the 2 treatment arms. Patients aged <2years of age were not enrolled in the placebo-controlled part of the study and were offered open label treatment in study PTC923MD-004-PKU.

Subjects who experienced a ≥ 15% reduction of blood Phe at the end of part 1 were considered responders and eligible for the part 2. Although not being a recognised definition for treatment responsiveness this cut-off was used with the purpose to create an enriched population for the placebo-controlled part of the pivotal study.

The dosage was chosen based on the results of the phase 2 study on adult subjects. There was no dedicated dose finding study in paediatric subjects. A popPK study using sparse and rich sampling from Study PTC923-MD-003-PKU and Study PTC923-MD-004-PKU by age group confirmed a lower exposure for patients <2 years and suggested that all patients would benefit form a fixed 60 mg/kg QD dosage regime. During the procedure, the Applicant expanded their data on patients <2years of age, including 8 patients <1 year of age (discussed under Study PTC923-MD-004-PKU below) and 25 patients ≥1 and <2 years. Patients treated with the currently used dose demonstrated consistent decreases in blood Phe levels, maintenance of blood Phe levels below target goal and tolerance of Phe dietary increases, in line with the results in older patients.

A forced dose escalation was proposed in part 2 of the study when subjects had been previously treated with 60 mg/kg during part 1, which is also the starting dose proposed in the SmPC. Updated data from the ongoing study including 65 naïve patients who started sepiapterin without dose escalation demonstrated that the proposed starting dose of 60 mg/kg is acceptable.

In part 1 of the study, after 14 days of sepiapterin treatment, 66.0% (103/156) of participants demonstrated a ≥30% reduction in blood Phe in response to sepiapterin, with a mean reduction of 462.2 µmol/L (65% reduction from baseline). Further, 114 (73.1%) of 156 participants demonstrated a ≥15% reduction in blood Phe level in response to sepiapterin. Positive results were also demonstrated in the 36 **cPKU patients** [45.7% (16/35) of participants demonstrated a ≥30% reduction from baseline blood Phe concentration with a mean reduction of 582.5 µmol/L (60% reduction from baseline)], participants that were documented as **nonresponsive to BH_4 therapy** [(n=56), 42.9% (24/56) responded to sepiapterin (≥30% reduction in blood Phe levels in baseline Phe, with a mean reduction in blood Phe concentration of 404.3 µmol/L (55% reduction from baseline)] and patients **receiving BH_4 therapy at the time of screening** [27 participants, 85.2% (23/27) responded to sepiapterin (≥30% reduction in blood Phe levels in baseline Phe), achieving a 48% reduction from the Phe concentration achieved on sapropterin therapy].

Considering the **primary analysis population** 89.8% (44/49) of the participants in the sepiapterin group achieved a reduction of ≥30% during Part 2 compared to 10.2% (5/49) in the placebo group. By Week 6, mean blood Phe concentration decreased significantly (decrease of 410.1 µmol/L, 63%), whereas Phe concentration remained relatively unchanged with placebo (decrease of 16.2 µmol/L, +1.4%). In the **FAS**, a statistically significant ($p<0.0001$) difference in the mean change in blood Phe levels from baseline to Weeks 5 and 6 was observed in the sepiapterin arm following treatment with sepiapterin compared with placebo. By Week 6, mean blood Phe levels decreased significantly in the sepiapterin arm (LS mean change of -289.59 µmol/L), whereas Phe levels remained relatively unchanged in the placebo arm (LS mean change of 65.31 µmol/L).

Results were more impressive in the previously determined responder sub-population but nevertheless were seen in FAS. There were no influence of sex and age on treatment effects.

Approximately 90% of subjects achieved their age-appropriate European target blood Phe concentration, and 22% of subjects achieved Phe concentration that are considered normalized.

The study was not sufficiently powered to detect differences based on the subpopulations and results should be read in light of this limitation.

Results from subgroup analyses should be read considering they were compromised by lack of power.

cPKU patients, known to have a less impressive response to treatments also showed an overall very good response in terms of Phe serum levels after the 6 weeks treatment (n=19; 63% lowering of blood Phe concentrations versus baseline); of the 15 with cPKU that had a Phe reduction in part 1 of >30%, significant (p<0.0001) larger reduction in mean blood Phe was seen in subjects with cPKU on sepiapterin *vs* placebo (-523.46 µmol/L vs -42.13) µmol/L), representing a 69% decrease from baseline. The study was not sufficiently powered to detect differences based on the subpopulations, the sample size was limited for each subgroup comparison and type I error was not controlled for these evaluations which are not considered in the multiplicity adjustment procedure. Consequently, reference to these descriptive results in the SmPC was updated and is considered acceptable with no reference p-value.

The proportion of **participants with baseline Phe ≥600 µmol/L, ≥360 µmol/L and Phe ≥120 µmol/L who achieved Phe levels below the defined threshold** in Part 2 were consistently significantly greater (p<0.0001) following treatment with sepiapterin compared with placebo.

Treatment efficacy was also demonstrated in subjects who were documented as **nonresponsive to BH_4**.

Patients that were treated with sapropterin at study screening, were also randomized after part 1 of the study. The improvement was even more significative with a longer treatment period, with patients on placebo returning to pre-study Phe levels. A significant (p<0.0001) difference in the mean change in blood Phe concentration from baseline to Weeks 5 and 6 was demonstrated with sepiapterin (n=14) compared with placebo (n=7). At Week 6, in subjects who received sepiapterin, mean blood Phe concentration decreased significantly (decrease of 438.28 µmol/L, 63%).

A **decrease in Phe:Tyr ratio** has been demonstrated following a decrease in Phe and no changes in Tyr levels. This decreased Phe:Tyr is associated with improved neuropsychological outcome.

Study PTC923-MD-004-PKU is an ongoing study evaluating the long-term safety of sepiapterin as well as the impact of sepiapterin treatment in changes from baseline in dietary Phe/protein consumption.

Currently presented results are descriptive only and result from an interim analysis conducted to provide data to support regulatory submission needs.

Although the study will include *de novo* patients, currently presented results (interim analysis) concern only patients that rolled from study PTC923-MD-003-PKU.

Study enrols both patients naïve to other than restrict diet treatment and patients treated with sapropterin or pegvaliase, with similar inclusion criteria to study PTC923-MD-003-PKU.

For all study subjects, the treatment phase consists of open-label daily treatment with sepiapterin for a minimum of 12 months. Proposed doses are consistent to the ones used in previous trial(s).

The study will also have a 26-week Dietary Phe Tolerance Assessment (for those patients achieving/having Phe <360 µmol/L after the first month of treatment). These patients will have every 2 weeks Phe dose increments (as per pre-defined protocol) according to previous Phe blood levels, up to the maximum protein intake tolerated.

The Applicant was asked to provide data in patients aged below 2 years old. In its D120 response, the Applicant provided updated data (data cut-off date 02 September 2024). Among the 12 patients aged below 2-year-old who were only treated in study PTC923-MD-004-PKU, 9 (66.7 %) achieved a ≥15% reduction in blood Phe level during the first 2 weeks of treatment and were considered responders.

Daily use of sepiapterin permitted an approximately 2.3-fold increase in mean daily Phe consumption (27.6 mg/kg/day at baseline versus 62.5 mg/kg/day at Week 26). The vast majority of participants achieved significant increases in dietary protein/Phe intake over the course of the Dietary Phe Tolerance Assessment, while mean blood Phe did not exceed the target of 360 µmol/L by Week 26.

The percentage of responders was comparable with the one observed in patients aged > 2 years old (73.9%). All 9 patients who were eligible to continue the study underwent the Dietary Phe Tolerance Assessment (primary efficacy endpoint). Least squares [LS] mean change from baseline to week 26 in Dietary Phe consumption between participants <2 and ≥2 years of age were 66.314 and 34.993 mg/kg/day, respectively. Results at Week 26 indicated an increase in dietary Phe consumption versus baseline while maintaining a blood Phe level < 360 µg/L in patients < 2 years in line with what was observed in patients aged > 2 years old. However, at this stage, data was only available for 4 patients aged <2 years.

Following a new request for updated data, the Applicant updated the number of patients < 2 years of age to 33 (previously 15), 8 aged <1 year. An analysis per age group showed a similar response to sepiapterin, with slightly lower results in the younger ages (mainly in the 1-2 year old group). Nevertheless, the results are consistent with results seen in older patients. A similar proportion of patients also achieved the goal serum Phe threshold levels by Week 2. Treated patients had their blood Phe levels under the goal limit for more than 24 months.

Of the 33 patients, 8 completed the 26 weeks Dietary Phe Tolerance Assessment, with 7 other still ongoing. Those patients tolerated a similar increase in Phe ingestion at week 26, when compared to older patients, consistently keeping Phe levels below the target goal.

Interim results for the 7 patients still ongoing are also discussed, being similar to the ones that already completed the challenge.

Out of the 8 patients that completed the Dietary Phe Tolerance Assessment, 3 were aged <1 year with similar results to patients 1-<2 years. Interestingly the change from baseline in term of mg/kg/day was higher in patients <2 years compared to the older ones, perhaps related to the fact that younger patients have more controlled diets and higher margin of progression in terms of tolerance.

In the overall population of subjects from Study PTC923-MD-003-PKU and the set of subjects who received sepiapterin in Part 2 of Study PTC923-MD-003-PKU, blood Phe remained generally unchanged throughout Study PTC923-MD-004-PKU as of the data cut-off date. Subjects who received placebo in Part 2 of Study PTC923-MD-003-PKU had a marked decrease in blood Phe levels after transition into Study PTC923-MD-004-PKU but according to data provided, in average, they had Phe blood levels consistently above the target threshold between month 6 and 16. Although, as stated, these are still preliminary results this needs further clarification.

Overall and in the Dietary Phe Tolerance Analysist Set, blood Tyr and median Phe:Tyr ratio remained generally constant through the end of the study. Some variations in later months occurred and will be further clarified upon full data analysis.

The final CSR for this study will be submitted once available via a Type-II variation post-authorisation.

Study PKU-002 (completed) was a Phase 2, randomized, multicenter, double-crossover, open label, active-controlled, study in adult subjects with PKU. This study assessed the efficacy of 7 days of treatment with 2 doses of sepiapterin comparing with the standard dose of sapropterin. A secondary objective of this study was to establish the dose to be used in future Phase 3 studies in PKU and to assess safety and tolerability of 2 doses of CNSA-001 in PKU patients.

Treatment with sepiapterin (irrespective of the dose) was superior to sapropterin, with sepiapterin at doses 20 mg/kg and 60 mg/kg/day resulting in a significant dose-dependent decrease in blood Phe concentration relative to baseline.

Pairwise comparisons of LSM blood Phe concentration reductions for all treatments' periods favoured sepiapterin 60 mg/kg/day.

In subjects with classical PKU (11/25 [45.8%]), treatment with sepiapterin (60 mg/kg/day) resulted in a significant decrease in blood Phe concentration relative to baseline (p=0.0287). Significance was not achieved with the other 2 treatments.

The submitted studies demonstrate sepiapterin's efficacy in treating patients with PKU:

- Showing significant and sustained decreases in Phe levels in patients at different levels of baseline Phe blood levels (disease severity) and in the different target ages. Majority of patients achieved Phe blood levels below the clinically agreed "controlled disease" threshold and a significant number of patients reach "normal" Phe levels.
- A dose treatment-effect decrease in Phe has been demonstrated.
- A better metabolic control was possible even with a more relaxed diet which has a huge impact on patient's quality of life.
- An improved metabolic control was achieved in some PKU patients otherwise not responsive or poor responsive to available treatment options: patients not responding to sapropterin and patients ineligible to treatment with pegvaliase that can rely solely on diet restriction.
- Based on the methodology that was followed, only patients who experienced a ≥ 15% reduction of blood Phe levels after receiving 60 mg/kg/d of sepiapterin for 14 days were included in pivotal study. Based on this a proposal for amendment of section 4.2 of the SmPC has been made stating that no controlled efficacy and safety data is available in patients who do not experience a reduction of 15% or greater reduction in blood Phe levels after receiving sepiapterin for 14 days.
- Although not being powered to generate significant evidence, benefit was also demonstrated in a higher proportion of classic PKU patients known to be more challenging to treat (expected to be more resistant to sapropterin or sepiapterin).

2.6.7. Conclusions on the clinical efficacy

Altogether, based on its mechanism of action and the submitted data of clinical trial results, sepiapterin may represent a significant improvement of the treatment of PKU patients and is considered to fulfil some of the current needs as:

1. Sepiapterin treatment was associated with significant and sustained decreases in Phe levels in patients at different levels of baseline Phe blood levels (disease severity) both in patients > 2 and 1 to <2 years old. Majority of patients achieved Phe blood levels below the clinically agreed "controlled disease" threshold and a significant number of patients reach "normal" Phe levels. In addition, facing the inclusion criteria defined for part 2 of the pivotal trial (patients who experienced a ≥ 15% reduction of blood Phe levels after receiving 60 mg/kg/day of sepiapterin for 14 days) section 4.2 reflects the lack of controlled efficacy and safety data in patients who do not experience a reduction of 15% or greater reduction in blood Phe levels after receiving sepiapterin for 14 days.
2. Sepiapterin treatment allowed for a higher Phe dietary consumption based on preliminary data from an ongoing study (pending confirmation with final trial results). A new update on the available data since submission of this ongoing study was submitted and supports the indication in patients aged <2 years. Available information on QoL endpoints was also submitted and demonstrate, consistently, in the different domains that were assessed, the benefit of the treatment with sepiapterin.
3. Supportive data of higher efficacy over sapropterin was generated
    a. Sepiapterin improved clinical response in patients responding to sapropterin
    b. Sepiapterin is superior to sapropterin when comparing
    c. efficacy results (larger responder rate and higher proportion of patients achieving the target Phe levels)
    d. Sepiapterin was efficacious in patients not responding to sapropterin
4. Sepiapterin offers an alternative to classic PKU patients <16 years of age (not eligible to be treated with pegvaliase) and whom respond insufficiently to sapropterin.

2.6.8. Clinical safety

The safety of sepiapterin was evaluated in three clinical studies in subjects with phenylketonuria (PKU):

- A completed, pivotal, Phase 3, placebo-controlled efficacy and safety study (PTC923-MD-003-PKU)
- An ongoing, Phase 3, long-term, open-label, efficacy and safety study (PTC923-MD-004-PKU)
- A completed, Phase 2, head-to-head comparator study of sepiapterin and sapropterin (PKU-002)

Data from PTC923-MD-003-PKU (completed) and PTC923-MD-004-PKU (ongoing; data cut-off: 22 September 2023) were pooled to perform an integrated analysis of safety. The PKU-002 study in the PKU population is not included in the safety analysis set, due to inclusion of patients exposed to sapropterin.

An updated analysis of the pooled data was provided for participants exposed to sepiapterin in Studies PTC923MD003PKU and PTC923MD004PKU (data cut-off date 02 September 2024). In this updated Pooled Safety Analysis Set, 222 participants have been exposed to at least 1 dose of sepiapterin, with total exposure

to sepiapterin of 192 patient-years. A total of 128 participants (57.7%) received sepiapterin for at least 26 weeks, and 95 participants (42.8%) received sepiapterin for at least 52 weeks. Whereas the original MAA pooled safety analysis included 10 participants aged <2 years, the current pooled analysis includes 15 participants aged <2 years.

Safety data from studies in 2 additional indications and from clinical pharmacology studies are provided at the study level as follows:

- 2 completed studies in indications other than PKU:
  - Diabetic gastroparesis (GAS-001)
  - Primary tetrahydrobiopterin (BH_4) deficiency (PBD-001)
- 5 completed clinical pharmacology and drug-drug interaction (DDI) studies in heathy volunteers:
  - First-in-human, single and multiple ascending doses, pharmacokinetic (PK)/pharmacodynamic (PD), and food effect study (PKU-001)
  - Bioavailability and food effect study (PTC923-MD-005-HV)
  - Japanese ethno-bridging and food effect study (PTC923-MD-007-HV)
  - Absorption, metabolism, and excretion study (PTC923-MD-008-HV)
  - DDI study (PTC923-DDI-101-HV)

Since these studies were not conducted in subjects with PKU, safety data from these studies are presented at the study level and are not included in the pooled safety analysis.

The overall safety conclusions from studies of sepiapterin evaluated in this Summary of Clinical Safety (SCS) are the following:

- Sepiapterin was well tolerated in patients with PKU across all age groups, genders, races, and ethnicities evaluated at an oral dose of up to 60 mg/kg/day and during long-term continuous treatment (exposures of up to 20 months).
- A total of 529 subjects have been exposed to at least 1 dose of sepiapterin across all clinical studies as of the data cut-off date of 02 September 2024. Exposure to sepiapterin across all clinical studies consists of 197.56 patient-years, including 194.82 patient-years of exposure in subjects with PKU (193.13 patient-years in the pooled safety database from Studies PTC923-MD-003-PKU and PTC923-MD-004-PKU).
  - PK data after the data cut-off date of 22 September 2023 from 2 paediatric subjects aged <2 years enrolled in Study PTC923-MD-004-PKU has been submitted, as per agreement with PDCO. No safety data from these 2 subjects have been included in this SCS, and these 2 subjects are not included in the summary of exposure across studies.
- The most commonly reported treatment-emergent adverse events (TEAEs) reported by more than 10% of subjects with PKU who received sepiapterin in Studies PTC923-MD-003-PKU and PTC923-MD-004-PKU were upper respiratory tract infection, headache, and diarrhoea.
- No deaths have been reported in any clinical study of sepiapterin.
- No serious TEAEs related or possibly related to study treatment have been reported.

- One serious TEAE has been reported in subjects with PKU: an event of asthmatic crisis in a patient with an ongoing history of asthma. The asthmatic crisis was considered severe, unrelated to study treatment, and resolved.
  - No serious TEAEs occurred in subjects with primary BH_4 deficiency (PBD) or in healthy subjects.
  - Two serious TEAEs occurred in subjects with diabetic gastroparesis including 2 events of impaired gastric emptying (1 moderate and 1 severe), both of which resolved and were not considered related to study drug.
- In total, 3 subjects with PKU discontinued from a study prior to completion due to TEAEs.
  - In Study PTC923-MD-003-PKU, 1 subject discontinued due to anxiety (mild) and 1 subject discontinued due to vomiting (mild); both of these events were considered possibly related to study treatment.
  - In Study PTC923-MD-004-PKU, 1 subject discontinued due to mild constipation and flatulence, and moderate disturbance in attention and headache. All 4 of these TEAEs were considered to be related to study treatment.
  - No TEAEs were reported that led to discontinuation in either healthy subjects or subjects in studies in other indications.
- In general, changes in clinical laboratory parameters, vital signs, physical examinations, and electrocardiograms (ECGs) in subjects with PKU were not clinically significant.
- The safety profile of sepiapterin in other indications and in healthy volunteers in clinical pharmacology studies was generally consistent with the safety observed in subjects with PKU.

The overall package of safety data indicates a consistent and favourable overall benefit-risk balance for sepiapterin in the treatment of patients with PKU.

The safety profile of sepiapterin is favourable, with no deaths or TEAEs related or possibly related to study treatment reported. In total, 3 subjects with PKU discontinued from a study prior to completion due to TEAEs but apparently none was related with the treatment, and they were reversible.

2.6.8.1. Patient exposure

Cumulatively, a total of 529 subjects have been exposed to sepiapterin in the completed clinical studies as of the data cut-off date of 02 September 2024. This includes 275 subjects with PKU, 10 subjects with moderate to severe diabetic gastroparesis, 8 subjects with PBD, and 236 healthy volunteers.

Exposure to sepiapterin across all clinical studies consists of 197.56 patient-years, including 194.82 patient-years of exposure in subjects with PKU (193.13 patient-years in the pooled safety database from Studies PTC923-MD-003-PKU and PTC923-MD-004-PKU). A total of 130 subjects have completed 6 months of treatment, and 95 subjects have completed 12 months of treatment.

Table 18. Summary of sepiapterin exposure in all clinical trials

Dose Regimen	Number of Subjects
Single dose (mg/kg)	
2.5	6
7.5	6
10	12
20	18[a,b]
40	30
60	85[a,b]
80	6
4000 mg (containing ~100 µCi ^{14}C-sepiapterin)	8
Multiple doses (mg/kg/day)	
5 for up to 1 week	6
10 for up to 1 week	4[c]
20 for up to 1 week	10[d,e]
20 for up to 2 weeks	10
30 for up to 2 weeks	3
60 for up to 1 week	30[e]
60 for up to 2 weeks	154[f]
Total dosed subjects	**388**

Abbreviations: CSR, clinical study report

[a] The 32 subjects who received a single dose of 60 mg/kg sepiapterin in Study PTC923-MD-005-HV also received a single dose of 20 mg/kg sepiapterin. These subjects are only counted in the 60 mg/kg category as the highest dose received.

[b] The 29 subjects who received a single dose of 60 mg/kg sepiapterin in Study PTC923-DDI-101-HV also received a single dose of 20 mg/kg sepiapterin. These subjects are only counted in the 60 mg/kg category as the highest dose received.

[c] The 4 subjects who received 10 mg/kg/day sepiapterin for 1 week in Study PBD-001 also received 2.5 mg/kg/day sepiapterin for 1 week. These subjects are only counted in the 10 mg/kg/day for 1 week category as the highest continuous dose received.

[d] The 4 subjects who received 20 mg/kg/day sepiapterin for 1 week in Study PBD-001 also received 5 mg/kg/day sepiapterin for 1 week. These subjects are only counted in the 20 mg/kg/day for 1 week category as the highest continuous dose received.

[e] The 24 subjects who were enrolled in Study PKU-002 received 20 mg/kg/day sepiapterin for 1 week and 60 mg/kg/day sepiapterin for 1 week. These subjects are only counted in the 60 mg/kg/day for 1 week category as the highest continuous dose received.

[f] Of the 154 subjects who received 60 mg/kg/day sepiapterin for up to 2 weeks, 44 only participated in Part 1 of Study PTC923 MD-003-PKU, 54 received sepiapterin in Part 1 of Study PTC923-MD-003-PKU then were randomized to placebo in Part 2, and 56 received 60 mg/kg/day sepiapterin in both Parts 1 and 2 of Study PTC923-MD-003-PKU. The 56 subjects who participated in both Parts 1 and 2 of Study PTC923-MD-003-PKU received 20 mg/kg/day for 2 weeks, then 40 mg/kg/day for 2 weeks, then 60 mg/kg/day for 2 weeks (6 weeks of treatment total). These subjects are only counted in the 60 mg/kg/day for 2 weeks category as the highest continuous dose received.

Note: Only the highest dose received per subject is listed for cases of escalating doses in individual studies.

In part 1 of the study PTC923 MD-003-PKU, 3 of 157 subjects were exposed to 30 mg/kg/day of sepiapterin. The 154 other subjects were exposed to 60 mg/kg/day of sepiapterin for two weeks. The overall mean [SD] exposure duration was 13.5 [2.25] days. In part 2, subjects were exposed to sepiapterin following a dose escalation from 20 to 60 mg/kg/day over 6 weeks. The overall mean [SD] exposure duration was 41.8 [2.05] days in the sepiapterin arm. Hence, the majority of included study patients completed study PTC923 MD-003-PKU as planned.

In study PTC923 MD-004-PKU (ongoing), 169 subjects had a mean [SD] study treatment exposure duration of 387.5 [242.15. Most subjects were exposed to 60 mg/kg/day. The mean duration of exposure at dose of 60 mg/kg was 401.5 [235.73]] days, which is relatively small, with regard to exposure in real life. The number of

subjects planned for this study was 200. The Applicant was asked to provide an update of the data for this extension study up to 38 months. Since the MAA pooled analysis, total exposure to sepiapterin has increased from 138 to 192 patient-years. A total of 128 participants (57.7%) have received sepiapterin for at least 26 weeks, and 95 participants (42.8%) have received sepiapterin for at least 52 weeks.

Exposure for each age group has increased as follows:

- <2 years of age: 4.0 to 7.5 patient-years
- 2 to <6 years of age: 14.9 to 22.0 patient-years
- 6 to <12 years of age: 34.1 to 46.2 patient-years
- 12 to <18 years of age: 38.2 to 51.4 patient-years
- ≥18 years of age: 47.1 to 64.5 patient-years

The exposed number of patients could be acceptable as it is a rare disease.

2.6.8.2. Adverse events

The most commonly reported TEAEs reported by more than 10% of subjects with PKU who received sepiapterin in Studies PTC923-MD-003-PKU and PTC923-MD-004-PKU were upper respiratory tract infection (21 [13.4%] subjects), headache (20 [12.7%] subjects), and diarrhoea (19 [12.1%] subjects)

- No deaths have been reported in any clinical study of sepiapterin.
- No serious TEAEs related or possibly related to the study treatment have been reported.
- One serious TEAE has been reported in subjects with PKU: an event of asthmatic crisis in a patient with an ongoing history of asthma. The asthmatic crisis was considered severe, unrelated to the study treatment, and resolved.
- In total, 3 subjects with PKU discontinued from a study prior to completion due to TEAEs.
  - In Study PTC923-MD-003-PKU, 1 subject discontinued due to anxiety (mild) and 1 subject discontinued due to vomiting (mild); both of these events were considered possibly related to the study treatment.
  - In Study PTC923-MD-004-PKU, 1 subject discontinued due to mild constipation and flatulence and moderate disturbance in attention and headache. All 4 of these TEAEs were considered to be related to the study treatment.
  - No TEAEs were reported that led to discontinuation in either healthy subjects or subjects in studies in other indications.
- In general, changes in clinical laboratory parameters, vital signs, physical examinations, and electrocardiograms in subjects with PKU were not clinically significant.

<u>Gastrointestinal effects</u>

Gastrointestinal effects are an identified risk of sepiapterin based on findings in the clinical development programme.

No significant gastrointestinal findings were reported in non-clinical studies.

In the Pooled PKU Studies (Study 003 and Study 004) of the 157 subjects treated with sepiapterin, 53 (33.8%) subjects experienced 94 Gastrointestinal disorder TEAEs. In the Pooled PKU Studies the most common treatment-related TEAEs by SOC in subjects who received any dose of sepiapterin were Gastrointestinal disorders (20.4%). The most common treatment-related TEAEs by preferred term (PT) with 4 or more subjects with events, were diarrhoea (6.4%), faeces discoloured (3.8%), nausea (3.2%), and abdominal pain upper (2.5%). The majority of these events were mild in severity. No SAEs were reported in the Pooled PKU Studies.

Diarrhoea:

In the Pooled PKU Studies, diarrhoea was the most common TEAE by PT under the Gastrointestinal disorders SOC. Of the 157 subjects treated with sepiapterin, 19 (12.1%) subjects experienced 21 diarrhoea TEAEs, with 11 TEAEs in 10 (6.4%) subjects considered to be treatment-related. All were non-serious and the majority were mild in severity; 18 (11.5%) subjects experienced Grade 1 TEAEs and there was 1 TEAE of Grade 2 severity in 1 (0.6%) subject. The majority of first diarrhoea TEAEs occurred during the initial 12 weeks of exposure to sepiapterin. Of the 54 subjects in the placebo group, 1 (1.9%) subject experienced 1 TEAE of diarrhoea, which was non-serious, of Grade 2 severity and not related to treatment. In Study 003, the majority of TEAEs of diarrhoea resolved; 2 subjects were reported to be recovering (Study 003).

In Study PKU-002, 1/24 (4.2%) subject experienced 1 TEAE of diarrhoea.

Faeces discoloured:

In the Pooled PKU Studies, of the 157 subjects treated with sepiapterin, 6 (3.8%) subjects experienced 10 TEAEs of faeces discoloured, all of which were considered to be treatment-related. All were non-serious and Grade 1 severity. All of the first TEAEs of faeces discoloured occurred during the initial 12 weeks of exposure to sepiapterin. Of the 54 subjects in the placebo group, none experienced a TEAE of faeces discoloured. In Study 003, the majority of TEAEs of faeces discoloured resolved; 2 subjects were reported to not have yet recovered (Study 003).

In Study PKU-002 no subjects experienced a TEAE of faeces discoloured.

Nausea:

In the Pooled PKU Studies, of the 157 subjects treated with sepiapterin, 7 (4.5%) subjects experienced 7 TEAEs of nausea, with 5 TEAEs in 5 (3.2%) subjects considered to be treatment-related. All were non-serious and Grade 1 severity. The first occurrence of nausea TEAEs was variable, with all of the nausea TEAEs occurring during the initial 26 weeks of exposure to sepiapterin. Of the 54 subjects in the placebo group, 3 (5.6%) subjects experienced 3 TEAEs of nausea of which 2 TEAEs in 2 (3.7%) subjects were considered treatment-related. All were non-serious and Grade 1 severity TEAEs. In Study 003, the majority of the TEAEs of nausea resolved; one subject was reported to have not yet recovered.

In Study PKU-002, 1/24 (4.2%) subject experienced a TEAE of nausea.

Abdominal pain / abdominal pain upper:

In the Pooled PKU Studies, of the 157 subjects treated with sepiapterin, 7 (4.5%) subjects experienced 7 TEAEs of abdominal pain, with 3 TEAEs in 3 (1.9%) subjects considered to be treatment-related. All were non-serious with 4 Grade 1 severity TEAEs reported in 4 (2.5%) subjects and 3 Grade 2 severity TEAEs in 3 (1.9%) subjects. All of the first TEAEs of abdominal pain occurred between the initial 2 to 26 weeks of exposure to sepiapterin. Of the 54 subjects in the placebo group, 1 (1.9%) subject experienced 1 TEAE of abdominal pain, which was

non-serious, Grade 1 severity and not considered treatment-related. In Study 003, all of the TEAEs of abdominal pain resolved.

In the Pooled PKU Studies, of the 157 subjects treated with sepiapterin, 6 (3.8%) subjects experienced 6 TEAEs of abdominal pain upper, with 4 TEAEs in 4 (2.5%) subjects considered to be treatment-related. All were non-serious and Grade 1 severity. All of the first TEAEs of abdominal pain upper occurred during the initial 12 weeks of exposure to sepiapterin. Of the 54 subjects in the placebo group, 1 (1.9%) subject experienced 1 TEAE of abdominal pain upper, which was non-serious, Grade 1 severity and not considered treatment-related. In Study 003, all of the TEAEs of abdominal pain upper resolved.

In Study PKU-002 no subject experienced a TEAE of abdominal pain or abdominal pain upper.

Overall, in the Pooled PKU Studies, 2 subjects receiving a dose of 60 mg/kg sepiapterin, discontinued treatment due to 3 gastrointestinal TEAEs. One subject in Part 1 of Study 003, experienced mild vomiting that resolved the same day, was non-serious, and was considered possibly related to study treatment. The second subject, in Study 004, discontinued the study on Day 8 due to TEAEs of constipation and flatulence, both mild in severity; and disturbance in attention and headache, both moderate in severity. All 4 of these TEAEs were non-serious and considered to be related to study treatment and were ongoing as of the data cutoff date.

Gastrointestinal effects are frequently reported with sepiapterin use, with diarrhoea listed as a very common (≥1/10) adverse reaction and faeces discoloured and abdominal pain listed as common (≥1/100 to <1/10) adverse reactions in the SmPC section 4.8.

Gastrointestinal effects are not an important risk of sepiapterin as all the TEAEs observed were non-serious, with the majority mild in severity and resolved, and can be managed in clinical practice using standard of care.

Sepiapterin has a good profile of adverse events. The most common adverse events related with the treatment are the gastrointestinal effects, and they are usually mild and well tolerated.

These adverse events are well and clearly described in the SmPC as adverse reactions.

2.6.8.3. Serious adverse event/deaths/other significant events

No serious TEAEs related or possibly related to the study treatment have been reported.

One serious TEAE has been reported in subjects with PKU: an event of asthmatic crisis in a patient with an ongoing history of asthma. The asthmatic crisis was considered severe, unrelated to the study treatment, and resolved.

In total, 3 subjects with PKU discontinued from a study prior to completion due to TEAEs.

- In Study PTC923-MD-003-PKU, 1 subject discontinued due to anxiety (mild) and 1 subject discontinued due to vomiting (mild); both of these events were considered possibly related to the study treatment.
- In Study PTC923-MD-004-PKU, 1 subject discontinued due to mild constipation and flatulence and moderate disturbance in attention and headache. All 4 of these TEAEs were considered to be related to the study treatment.
- No TEAEs were reported that led to discontinuation in either healthy subjects or subjects in studies in other indications.

There were no deaths reported.

There are no reports of serious TEAEs apparently related with sepiapterin.

There are just 3 patients that reported discontinuation of treatment, but none apparently related with the treatment.

It is agreed that there is strong evidence of the safety profile.

2.6.8.4. Laboratory findings

In general, changes in clinical laboratory parameters, vital signs, physical examinations, and electrocardiograms in subjects with PKU were not clinically significant.

Data from nonclinical studies demonstrate a nonclinical safety profile supportive of the use of sepiapterin at the highest proposed dose of 60 mg/kg/day based on age for treatment of patients with PKU.

Nonclinical studies described in the literature support the mechanism of action of sepiapterin.

Secondary PD assessments performed in vitro showed that it is unlikely that sepiapterin would have any clinically meaningful off-target interactions. Sepiapterin has shown no adverse effects on CNS, respiratory, and CV function in nonclinical safety pharmacology studies. Sepiapterin had no discernible effects in the hERG channel study at the maximum concentration tested.

Sepiapterin belongs to a class of compounds with pharmacological activity that is not associated with drug dependence.

The PK (ADME) properties of sepiapterin have been assessed in multiple species (mice, rats, rabbits, dogs, marmoset monkeys, and cynomolgus monkeys). Following oral administration, sepiapterin was quickly absorbed (time to maximal observed concentration [T_{max}] generally ≤2 hours) and rapidly converted to BH_4 in all species studied.

Sepiapterin did not show signs of potential cytochrome P450 (CYP)-mediated metabolic DDIs during in vitro testing. The metabolism of sepiapterin and its major metabolite BH_4 is not mediated by CYP enzymes. *In vitro*, sepiapterin is a substrate and an inhibitor of efflux transporter BCRP, but not a substrate nor an inhibitor of multidrug resistance protein 1 (MDR1; also called P-glycoprotein [P-gp]); BH_4 is a substrate and an inhibitor of BCRP and a substrate and an inhibitor of MDR1. Clinical study in adult healthy volunteers demonstrated that sepiapterin at the oral dose 60 mg/kg did not inhibit BCRP substrate rosuvastatin and coadministration sepiapterin with BCRP inhibitor curcumin only slightly increased the major metabolite BH_4 C_{max} and $AUC^{0\text{-}24h}$ (~24%) and this increase was considered not clinically relevant.

Rats and marmoset monkeys were selected as the toxicology species for studies of sepiapterin because, similar to humans, absorption of sepiapterin in these species was fast, and the conversion from sepiapterin to BH_4 was rapid and extensive. In the systemic circulation, levels of sepiapterin were less than 5% of the BH_4 values in rats and marmoset monkeys.

The only target organ identified in the 13- and 26-week studies in Sprague Dawley rats was the kidney at doses ≥100 mg/kg/day. All findings were partially or fully reversible during the recovery phases. No sepiapterin-related renal changes were observed in either the 13-week (300 mg/kg/day) or 9-month (300 mg/kg/day) studies in marmoset monkeys.

Sepiapterin was not genotoxic in the *in vitro* bacterial mutation assay or in the in vivo micronucleus and comet assays in rats.

There were no adverse effects noted in the reproductive and developmental toxicology studies performed with sepiapterin. Sepiapterin had no maternal or paternal toxicity or effects on male or female mating or fertility parameters or any effects on any reproductive parameters in Sprague Dawley rats at doses up to 300 mg/kg/day (highest tested dose). No maternal or EFD toxicity was observed in pregnant rat and rabbit studies at doses up to 1000 mg/day. However, in pregnant rabbits, there were nonadverse, transient mean maternal body weight loss and decreased mean food consumption at the beginning of dosing (gestational day [GD] 7 to 10) at 1000 mg/kg/day, the highest tested dose. In the pre-and post-natal development study in rats, maternal doses of sepiapterin up to 300 mg/kg/day (highest tested dose) were well tolerated in the F0 generation females and did not affect growth or development of the F1 generation rats during the preweaning or postweaning periods. There were no effects on reflex and physical development evaluations, sexual maturation, or neurobehavioral or reproductive function in the F1 generation rats. No toxicities were observed in any of the reproductive organs in the chronic studies up to 26 weeks in rats and 39 weeks in marmoset monkeys. There were no adverse effects noted in the juvenile toxicity studies at doses up to 30/300 mg/kg/day from postnatal day (PND) 4 through PND70.

Sepiapterin did not demonstrate any phototoxic potential.

There is a potential risk of DDIs with inhibitors of DHFR (e.g., trimethoprim, methotrexate, pemetrexed, pralatrexate, and trimetrexate) based on known pharmacodynamics. However, the effect has not been investigated clinically (Module SVII.1.1).

There were no safety concerns identified based on non-clinical findings.

2.6.8.5. In vitro biomarker test for patient selection for safety

There are no *in vitro* biomarkers described.

2.6.8.6. Safety in special populations

Age

Overall, no differential profile was identified from the examination of TEAEs by age (1 to 61 years of age). Study PTC923-MD-004-PKU is ongoing and actively recruiting patients with PKU of all ages, including those <28 days. At the time of the data cut-off, 3 patients <2 years of age have been enrolled and no patients <28 days have been included.

As observed in the adult population, the majority of treatment-related TEAEs were gastrointestinal disorders (Module 2.7.4, Section 5.1.1). The majority of treatment-related TEAEs occurred at the 60 mg/kg/day dose. Furthermore, at 60 mg/kg/day, the frequency of treatment-related TEAEs in children was lower than that in the adults at an equivalent dose, consistent with a lack of subgroup effect of age on the incidence or severity of TEAEs in sepiapterin-treated subjects.

While the safety of sepiapterin has been evaluated in paediatric patients with PKU aged 1 to 17 years, as stated in the SmPC Section 4.2, frequent blood monitoring is recommended to ensure adequate blood Phe level control.

Sepiapterin has not been studied in subjects over 65 years of age. As stated in the SmPC Section 4.2, caution should be exercised when prescribing in older age.

Sex, Race, and Ethnicity

The incidence and nature of TEAEs at System Organ Class and Preferred Term level TEAE profile of sepiapterin was consistent across the predefined subgroups (sex, race, and ethnicity) in patients with PKU, consistent with a lack of subgroup effect on the incidence or severity of TEAEs in sepiapterin-treated subjects (Module 2.7.4, Sections 5.1.2, 5.1.3, and 5.1.4, respectively).

Renal and Hepatic Impairment

Across the completed clinical studies, there were no TEAEs or clinically significant laboratory findings suggestive of hepatic or renal impairment. Sepiapterin is rapidly absorbed and converted to BH_4 via a 2-step process, mediated by SR and DHFR. The metabolism is not mediated by CYP450 enzymes, which is related to hepatic impairment. Additionally, the fraction of the dose eliminated via renal clearance is very small. Part A of Study PTC923-RI-103-HV (Cohorts 1 and 2; severe renal impairment and normal renal function, respectively) and Stage A of Study PTC923-HI-104-HV (Groups 1 and 3; normal hepatic function and moderate hepatic impairment, respectively) are currently nearing completion and data are not yet available. As dedicated studies to assess the effect of renal impairment (PTC923-RI-103-HV) and hepatic impairment (PTC923-HI-104-HV) on the pharmacokinetics (PK) and safety of sepiapterin and the active metabolite BH_4 are ongoing, the Applicant committed to submit a Type-II variation post-authorisation to include data upon completion. Meanwhile, as stated in the SmPC Section 4.2, caution should be exercised when prescribing to patients with either renal or hepatic impairment.

Pregnancy and Lactation

There are limited data from the use of sepiapterin in pregnant woman. In non-clinical studies, there was no indication of direct or indirect harm with respect to pregnancy, early embryonic development, embryo-foetal development, pre- and post-natal development; there have been no adequate and well-controlled studies with sepiapterin in pregnant women. As stated in the SmPC, caution should be exercised when prescribing to pregnant women.

Although female patients who were pregnant or considering pregnancy, was an exclusion criterion in Study 003 (Module SIV.1), there has been one pregnancy reported in the clinical study programme. The subject was enrolled in Study 003. The subject received sepiapterin 60 mg/kg for 15 days during Part 1 of the study. Thirty days after starting study drug, the subject's repeat urine pregnancy test and serum pregnancy test confirmed the pregnancy. The subject was withdrawn from the study due to pregnancy and did not enter Part 2 or the open-label extension period of the study. The outcome of the pregnancy was normal.

There are insufficient data to assess the presence of sepiapterin in human milk, and there are no data on the effects on milk production. As stated in the SmPC, caution should be exercised when using sepiapterin during breastfeeding.

Table 19. Exposure of special populations included or not in clinical trial development programmes

Type of Special Population	Exposure
Pregnant women	Although female patients who were pregnant or considering pregnancy, was an exclusion criterion in Study 003 (Module SIV.1), there has been one pregnancy reported in the clinical study programme. The subject was enrolled in Study 003. The subject received sepiapterin 60 mg/kg for 15 days during Part 1 of the study. Thirty days after starting study drug, the subject's repeat urine pregnancy test and serum pregnancy test confirmed the pregnancy. The subject was withdrawn from the study due to pregnancy and did not enter Part 2 or the open-label extension period of the study. The outcome of the pregnancy was normal.
Breastfeeding women	Not included in the clinical development programme.
Patients with relevant comorbidities: • Patients with hepatic impairment	Sepiapterin has not been studied in patients with hepatic impairment.
• Patients with renal impairment	Sepiapterin has not been studied in patients with renal impairment.
• Patients with cardiovascular impairment	There is limited exposure in patients with cardiovascular impairment. There was 1 (2.1%) subject in Part 1 of Study 003 with a medical history of heart murmur under the Investigations SOC (Study 003 CSR, Table 14.1.8.1, Listing 16.2.4.6). Additionally, there was 1 (0.6%) subject in Part 2 of Study 003 with a medical history of tachycardia and palpitations (under the Cardiac disorders SOC) (Study 003 CSR, Table 14.1.8.1, Listing 16.2.4.6). This subject received placebo in Part 2 of Study 003.
• Immunocompromised patients	Sepiapterin has not been studied in immunocompromised patients.
• Patients with a disease severity different from inclusion criteria in clinical trials	In Study 003, subjects with any phenylalanine hydroxylase mutation were permitted to screen and enrol into the study. However, subjects with classical PKU (ie, blood Phe birth levels ≥1200 µmol/L and/or historical evidence of Phe concentrations ≥1200 µmol/L in their medical history) were to be capped at 20% of the total study population. In Part 2 of Study 003, PKU disease characteristics were generally similar between the sepiapterin and placebo groups. More than half (65.5%) of the 110 subjects had PKU diagnosed at birth, and the majority (82.7%) had "biochemically defined" nonclassical PKU (Study 003 CSR, Table 14.1.6.1).

Type of Special Population	Exposure
Population with relevant different ethnic origin	From the pooled exposure data (Module SIII, Table 7), the main ethnic groups for subjects with PKU treated with sepiapterin was Not Hispanic or Latino (153 of 181), with smaller numbers of subjects characterised as Hispanic or Latino (25 of 181), Not Reported (2 of 181), and unknown (1 of 181).
Subpopulations carrying relevant genetic polymorphisms	Subjects with any phenylalanine hydroxylase mutation were permitted to screen and enrol into Study 003. Genotyping was not required for inclusion/exclusion; however, all subjects in Study 003 underwent genotyping unless documented in their medical history and this data was collected for analysis.
Use in paediatric patients	From the pooled exposure data (Module SIII, Table 4), 66.67% of male subjects and 44.32% of female subjects with PKU treated with sepiapterin were <18 years of age. The number of paediatric subjects with PKU treated with sepiapterin were as follows: <2 years: 3 of 181 2 to <6 years: 15 of 181 6 to <12 years: 40 of 181 12 to <18 years: 43 of 181 Study PKU-002 did not include paediatric subjects. The inclusion criteria specified patients ≥18 years and ≤60 years of age.

Abbreviations: Phe, phenylalanine; PKU, phenylketonuria; SOC, System Organ Class.

There is insufficient information in children bellow 2 years, pregnancy and lactation.

From physiopathologic point of view no special risks are expected to be identified in these population but further data is requested before final conclusions can be made. The Applicant confirmed that one FuQ including both pregnancy and lactation will be used. The FuQ is designed to collect specific key information relating to both pregnancy and lactation. The FuQ is added as a routine pharmacovigilance activity for the missing information use during pregnancy and lactation to the EU-RMP version 0.3. The implementation of one FuQ covering both pregnancy and lactation is considered acceptable.

2.6.8.7. Immunological events

No immunological events were reported or suspected.

2.6.8.8. Safety related to drug-drug interactions and other interactions

Drug-drug interaction studies indicate that sepiapterin and the major circulating active moiety BH_4 are unlikely to be either perpetrators or victims of CYP450 enzyme or transporter mediated drug-drug interactions *in vivo*. As such, no dose adjustment would be required if inhibitors or substrates of CYP or transporter enzymes are coadministered with sepiapterin

Once dosed, sepiapterin is quickly converted to BH_4 by SR and DHFR unidirectionally (Smith 2019b). Carbonyl reductase may also be involved in the first step of sepiapterin reduction to form the intermediate metabolite, BH2 (Werner 2011). Increased BH4 from sepiapterin administration is presumed to be oxidized during catalytic aromatic amino acid hydroxylation and regenerated by pterin-4α-carbinolamine dehydratase and dihydropteridine reductase like endogenous BH_4.

Conversion/formation of BH_4 following sepiapterin oral administration may be subject to inhibition of DHFR due to inhibitors such as trimethoprim, methotrexate, pemetrexed, pralatrexate, and trimetrexate. No specific studies have been conducted to investigate the effects. As stated in the SmPC Sections 4.4 and 4.5, caution is recommended when using such medicinal products while taking sepiapterin. More frequent monitoring of blood Phe concentration may be required because these drugs may inhibit the enzymatic conversion of sepiapterin to BH_4.

The potential of drug interactions in the presence of SR inhibitors has not been investigated clinically. As stated in the SmPC Section 4.5, caution should be exercised when sepiapterin is coadministered with SR inhibitors, such as sulfasalazine or sulfamethoxazole.

Based on *in vitro* data, sepiapterin was not a substrate of efflux transporter multidrug resistance protein 1 (MDR1) or of uptake transporters organic anion transporting polypeptide (OATP)1B1, OATP1B3, multidrug and toxin extrusion (MATE)1, MATE2-K, organic anion transporter (OAT)1, OAT3, organic cation transporter (OCT)2, and equilibrative nucleoside transporter (ENT)1 at tested conditions. Sepiapterin was a substrate of ENT2 only at the highest tested concentration.

Sepiapterin did not inhibit the efflux transporters MDR1 or bile salt export pump or the uptake transporters MATE1, MATE2-K, OAT1, OAT3, OATP1B3, OCT1, and OCT2. Sepiapterin was a weak inhibitor of OATP1B1.

Based on *in vitro* data, BH_4 was a substrate and an inhibitor of BCRP and MDR1 BH_4 was a substrate of the MATE2-K uptake transporter only at the highest tested concentration and was unlikely to be a substrate of uptake transporters MATE1, OAT1, OATP1B1, OATP1B3, OCT1, and OCT2. BH_4 did not inhibit the efflux transporter BSEP or uptake transporters ENT1, ENT2, MATE1, MATE2-K, OAT1, OAT3, OATP1B1, and OATP1B3. BH_4 weakly inhibited OCT1 and OCT2.

In vitro, sepiapterin and BH_4 were both substrates and inhibitors of efflux transporter breast cancer resistant protein (BCRP). The potential of sepiapterin and BH_4 as victims or perpetrators of BCRP-mediated drug interactions was investigated clinically in adult healthy subjects in Study PTC923-DDI-101-HV

- Oral coadministration of curcumin, a BCRP inhibitor, and sepiapterin in healthy adults resulted in slight increases in average AUCs and C_{max} of BH_4 by approximately 20% to 24%, after a single dose. This increase is not clinically relevant, and no dose adjustment of sepiapterin is warranted.
- Oral coadministration of sepiapterin and rosuvastatin, a BCRP substrate, had no impact on rosuvastatin exposure, and no dose adjustment is warranted.

No clinically significant drug-drug interactions were detected; however, the potential of drug interactions in the presence of DHFR and SR inhibitors has not been investigated clinically. As stated in the SmPC Sections 4.4 and 4.5, caution should be exercised when using such medicinal products while taking sepiapterin.

Sepiapterin looks safe regarding in drug-drug interactions profile. There are potential interactions with DHFR and SR inhibitors. Caution is needed upon concomitant use of sepiapterin and DHFR and SR inhibitors as stated in the SmPC.

2.6.8.9. Discontinuation due to adverse events

In total, 3 subjects with PKU discontinued from a study prior to completion due to TEAEs.

- In Study PTC923-MD-003-PKU, 1 subject discontinued due to anxiety (mild) and 1 subject discontinued due to vomiting (mild); both of these events were considered possibly related to the study treatment.
- In Study PTC923-MD-004-PKU, 1 subject discontinued due to mild constipation and flatulence and moderate disturbance in attention and headache. All 4 of these TEAEs were considered to be related to the study treatment.

No TEAEs were reported that led to discontinuation in either healthy subjects or subjects in studies in other indications.

The discontinuation with sepiapterin was rare in the presented studies and none was apparently related with the treatment.

2.6.8.10. Post marketing experience

Sepiapterin has not yet been marketed in any country.

2.6.9. Discussion on clinical safety

Safety data collection

Sepiapterin was studied in three clinical studies in PKU subjects. For the safety, the study phase 3, randomized, double blind, placebo controlled (PTC923 MD-003-PKU) and the phase 3 (PTC923 MD 004-PKU) study open label, uncontrolled are pooled. The PKU-002 study in the PKU population is not included in the safety analysis set, due to inclusion of patients exposed to sapropterin.

An updated analysis of the pooled data was provided for participants exposed to sepiapterin in Studies PTC923MD003PKU and PTC923MD004PKU (data cut-off date 02 September 2024). In this updated Pooled Safety Analysis Set, 222 participants have been exposed to at least 1 dose of sepiapterin, with total exposure to sepiapterin of 192 patient-years. A total of 128 participants (57.7%) received sepiapterin for at least 26 weeks, and 95 participants (42.8%) received sepiapterin for at least 52 weeks. Whereas the original MAA pooled safety analysis included 10 participants aged <2 years, the pooled analysis includes 15 participants aged <2 years.

The TEAEs in the updated Pooled Safety Analysis Set were similar in type, severity, and frequency to those seen in the MAA safety analysis. Updated analyses of TEAEs by age group were consistent with the findings reported in the MAA. Upper respiratory tract infection was the most commonly reported TEAE in participants who received sepiapterin (44 participants [19.8%]). There were no apparent differences in overall rates of TEAEs or in the TEAE Preferred Terms (PTs) reported among age groups. The most common treatment-related TEAE by Preferred Term was diarrhoea (19 participants [8.6%]).

No deaths have occurred at any time in the sepiapterin clinical programme. In the updated Pooled Safety Analysis Set, 3 participants who received sepiapterin experienced treatment emergent SAEs since the MAA

data cut-off date, all of which resolved and none of which was considered related to sepiapterin. No additional participants discontinued from the study after the MAA data cut-off date.

In general, changes in clinical laboratory parameters, vital signs, physical examinations, and ECGs were not clinically significant.

Sepiapterin is safe and well tolerated and has a comparable safety profile between participants with PKU <2 and ≥2 years of age, indicating the safety is comparable across all age groups

The safety profile observed as of the 13 February 2025 data cut-off did not differ from that seen in the 02 September 2024 data cutoff presented in the D120 response.

In conclusion, sepiapterin was well tolerated in participants with PKU across all age groups (including those aged <2 years), during long-term continuous exposure of up to 31 months. There are no new safety signals and no changes to the proposed prescribing information. The safety profile continues to be positive for sepiapterin.

The safety of sepiapterin has also been assessed in 2 clinical studies in 2 other indications (diabetic gastroparesis and primary tetrahydrobiopterin [BH_4] deficiency), and in 5 clinical pharmacology studies in healthy volunteers. Since these studies were not conducted in subjects with PKU, safety data from these studies are presented at the study level and are not included in the pooled safety analysis.

For the following sections of the report, the data will be evaluated by study and not by pool.

Patient exposure

A total of 529 subjects have been exposed to sepiapterin in the completed clinical studies as of the data cut-off date of 02 September 2024. This includes 275 subjects with PKU, 10 subjects with moderate to severe diabetic gastroparesis, 8 subjects with Primary BH_4 deficiency (PBD), and 236 healthy volunteers.

In part 1 of the study PTC923 MD-003-PKU, 3 of 157 subjects were exposed to 30 mg/kg/day of sepiapterin. The 154 other subjects were exposed to 60 mg/kg/day of sepiapterin for two weeks. The overall mean [SD] exposure duration was 13.5 [2.25] days. In part 2, subjects were exposed to sepiapterin following a dose escalation from 20 to 60 mg/kg/day over 6 weeks. The overall mean [SD] exposure duration was 41.8 [2.05] days in the sepiapterin arm. Hence, the majority of included study patients completed study PTC923 MD-003-PKU as planned.

In study PTC923 MD-004-PKU (ongoing), 169 subjects had a mean [SD] study treatment exposure duration of 387.5 [242.15] days. Most subjects were exposed to 60 mg/kg/day. The mean duration of exposure at dose of 60 mg/kg was 401.5 [235.73] days, which is relatively small, with regard to exposure in real life. The number of subjects planned for this study was 200.

Patient and baseline characteristics

In study PTC923 MD-003-PKU, the majority of subjects (65.5%) who progressed to Part 2 (110) were <18 years of age (median of 14 years; range: 2 to 54). Protocol eligibility required no supplementation with BH_4 or pegvaliase-pqpz. Subjects on such therapies at screening had to wash out before Part 1.

More than half (51.0%) had used BH_4 before, one (0.6%) had used pegvaliase-pqpz, and 17.8% were currently on these supplements at screening. Of the 157 subjects, 35.7% were BH_4 non-responders, and in 37.6%, prior BH_4 treatment status was unknown.

In PTC923 MD-004-PKU, median age was 14 years (range: 2-54) and 66.3% of subjects were under 18.

Overview of TEAEs

In the pivotal study PTC923 MD-003-PKU part 1 (2 weeks of exposure), 43.3 % of patients experienced TEAEs. And in part 2, (6 weeks of exposure) there were more TEAEs in the sepiapterin arm than in the placebo arm (58.9% vs 33.3%). However, the treatment-related TEAEs were rather low and seemed similar between the sepiapterin and placebo arms (10.7% vs 11.1%). There were no serious TEAEs or TEAEs of Grade ≥3.

In study PTC923-MD-004-PKU (ongoing), there was one serious TEAE and 3 TEAEs ≥ grade 3.

Regarding the summary of treatment-emergent adverse events (TEAEs), no deaths were reported, and no treatment-related serious adverse events (SAEs) occurred. However, one unrelated SAE was observed, for which a narrative should be requested. There were no TEAEs leading to discontinuation of treatment or withdrawal from the study. 14 participants experienced TEAEs, of whom 4 had TEAEs.

Overall, these data are reassuring. However, the short period of time of treatment exposure hampers further assessment.

Common TEAEs

In the study PTC923 MD-003-PKU part 1, the most commonly reported TEAEs by PT were **diarrhoea** (8 [5.1%] subjects), **headache** and **upper respiratory tract infection** (7 [4.5%] subjects each). By SOC, the most commonly reported TEAEs were **gastrointestinal disorders** (30 [19.1%] subjects) and **Infections and Infestation** (24 [15.3%] subjects).

In part 2, in the sepiapterin arm, the most common TEAEs reported by SOC **were Infections and Infestations** (12 [21.4%] subjects) and **gastrointestinal disorders** (10 [18.5%] subjects) while the most common TEAEs in the placebo arm were gastrointestinal disorders (10 [18.5%] subjects).

The most common TEAEs by PT in the sepiapterin arm were **diarrhoea and headache** (4 [7.1%] subjects each). These TEAEs were higher in the sepiapterin group than in the placebo group: diarrhoea (7.1% vs 1.9) and headache (7.1% vs 1.9%).

In the study PTC923-MD-004-PKU, the most frequently reported TEAEs by SOC **were infections and infestations** (31 [29.8%] 58 subjects). By PT, the most frequently reported TEAEs were **upper respiratory tract infection** (13 [12.5%] 16 subjects), **headache** (12 [11.5%] 19 subjects), and **nasopharyngitis** (11 [10.6] 15 subjects). The majority of TEAEs were only reported in 1 subject.

Severity of TEAEs

In study PTC923 MD-003-PKU, all TEAEs in Part 1 and Part 2 were either mild or moderate in severity (ie, CTCAE Grade 1 or 2). No severe (CTCAE Grade 3 or higher) TEAEs were reported during the study.

In the study PTC923-MD-004-PKU, three subjects (2.9%) experienced severe TEAEs (anxiety, asthmatic crisis, endometriosis, and liver contusion), while no subjects experienced a Grade >3 TEAE. The severe TEAEs of asthmatic crisis and endometriosis were reported by the same subject. None of the severe TEAEs reported were considered as related to study treatment.

Serious TEAEs

No SAEs were reported in Study PTC923 MD-003-PKU or in Study PKU-002. There was 1 SAE reported in the ongoing Study PTC923-MD-004-PKU. This was an asthma crisis during a mild pneumonia infection. The SAE was considered severe and unrelated to study treatment.

In the other supportive clinical studies, there were 2 SAEs (2 impaired gastric emptying) but considered as not related to the study drug.

No deaths have occurred in any clinical study of sepiapterin.

TEAEs related to study drug

The Applicant provided the Preferred Terms (PTs) and narratives for cases with treatment-related TEAEs, which appeared clear and well valuated. The Applicant also provided narratives and an evaluation for the cases of pyrexia, which was not a common TEAE in participants over 2 years of age. Those narratives are supported, and we it is agreed that they appear to be correlated with more common infection in these age group.

In the study PTC923 MD-003-PKU part 1, the most frequently reported treatment-related TEAEs were **gastrointestinal disorders** (23 [14.6%]) subjects). The most commonly reported gastrointestinal disorders TEAEs were diarrhoea (6, 3.9%), faeces discoloured (4, 2.6%), vomiting, abdominal pain upper, and nausea (3, 1.9% each).

In the part 2, the occurrence of treatment related TEAEs were similar between the sepiapterin group and the placebo group (10.7% vs 11.1%). The most frequently reported treatment related TEAEs were gastrointestinal disorders (4 [7.1%] and 4 [7.4%] subjects in the sepiapterin and placebo arms, respectively). The most frequent treatment-related TEAE by PT in sepiapterin group was faeces discoloured with 3.6 % versus 0 % in the placebo group.

In the study PTC923-MD-004-PKU, the most frequently reported treatment-related TEAEs by SOC were gastrointestinal disorders (12 subjects [11.5%]). The most frequent treatment-related TEAE by PT was headache (6 subjects [5.8%]).

It should be noted that the other studies (PKU-002, for other indications and in healthy volunteers) do not provide any further information. There were no serious cases in these studies.

ADRs of special interest

The Applicant did not submit ADRs of special interest.

Discontinuation due to adverse event

In Study PTC923 MD-003-PKU, 2 subjects discontinued from the study in Part 1 due to mild non-serious TEAEs that were considered treatment related (severity anxiety and vomiting). The subject who experimented with vomiting withdrew from the study.

In Study PTC923-MD-004-PKU PKU (60 mg/kg dose group) a patient discontinued the study due to TEAEs of constipation, flatulence, disturbance in attention and headache. All 4 of these TEAEs were non-serious and considered to be related to study treatment.

Safety in special populations

Age:

The proportion of subjects with TEAEs appeared to be similar between the groups aged 2 to 6 years (73.3%) and 6 to 12 years (72.5%), as well as between the group aged 12 to 18 (67.4%) and over 18 years (64.3%). It appears to be a higher proportion of subjects with TEAEs in the < 12 years population than in the >12 years population.

There are more treatment-related TEAE in patients aged >18 years (33.9%) compared with other age groups (around 25%). There is no treatment related TEAE in patients < 2 years. However, interpretation is difficult due to small number of subjects in the youngest age group (3 compared to 15, 40, 43 and 56, respectively)

The most common PT in subjects aged 6 to <12 was Upper respiratory tract infection (25%).

Sex:

The proportion of subjects in sepiapterin arm with TEAEs appeared to be similar between male and female (65.9% and 72.2%). The same applies to treatment related TEAEs (28.2% in the male group and 27.8% in the female group).

The distribution of race (142 white vs. 15 non-white) and ethnicity (25 Hispanic or Latino vs. 129 Not Hispanic or Latino) subgroups was not uniform, there is no evidence of harmful effects depending on subgroups.

Across the completed clinical studies, there were no TEAEs or clinically significant laboratory findings suggestive of hepatic or renal impairment. However, the safety of sepiapterin in patients with renal or hepatic insufficiency has not been established. Dedicated studies to assess the effect of renal impairment (PTC923-RI-103-HV) and hepatic impairment (PTC923-HI-104-HV) on the pharmacokinetics (PK) and safety of sepiapterin and the active metabolite BH_4 are ongoing, a Type-II variation will be submitted post-authorisation to include data upon completion.

Interpretation of this section is difficult because the results are pooled and include placebo. The Applicant was therefore asked to provide tables for each separate study. The Applicant provided tables for each separate study and pooled studies showing AEs overall and by age group, excluding those participants who received placebo in Part 2 of Study PTC923-MD-003-PKU from the sepiapterin arm as requested.

The proportion of subjects with TEAEs in the <2 years group appeared higher than in the other age groups (93.3% vs. 77.3% in the 2–6 years group, 67.6% in the 6–12 years group, 63.9% in the 12–18 years group, and 70.5% in the >18 years group).

Treatment-related TEAEs in the <2 years group appear similar to those in the other groups (26.7%), whereas in patients aged >18 years there are more treatment-related TEAEs (44.4%) compared with other age groups (between 25% and 30%). The most common PT in subjects aged <2 years are diaper dermatitis, discoloured faces, and vomiting. The Applicant was requested to provide the two cases of diaper dermatitis. The Applicant provided data about cases of diaper dermatitis and stated that both events reported have been considered to be unlikely related to study drug since diaper dermatitis is an exceptionally common finding in non-toilet-trained infants/toddlers and is even more common with a medical history of eczema. Rashes or dermatitis has been no signed as safety signal across the rest of the sepiapterin data.

Drug-drug interactions

BH_4 is also a cofactor of Tyrosine Hydroxylase. According to the SmPC of the sapropterin, cases of convulsion, exacerbation of convulsion, increased excitability and irritability have been observed during co-administration of levodopa and sapropterin in BH_4-deficient patients.

BH_4 is a cofactor for nitric oxide synthetase. Caution is recommended during concomitant use of Sepiapterin with all medicinal products that cause vasodilation, including those administered topically, by affecting nitric oxide (NO) metabolism or action including classical NO donors (e.g. glyceryl trinitrate (GTN), isosorbide dinitrate (ISDN), sodium nitroprusside (SNP), molsidomin), phosphodiesterase type 5 (PDE-5) inhibitors and minoxidil.

Given the mechanism of action of sepiapterin, the Applicant was asked to add in the SmPC the interaction with NO donors and levodopa. According to the Applicant, the risk of PD interaction with NO donors is limited at the therapeutic sepiapterin dose. Fifty-four (54) patients included had taken any drugs potentially causing vasodilation, and there were no observed AEs related to this potential PD interaction as hypotension. However, the current available data are not convincing enough to discard this risk at the post-marketing level. Given that sepiapterin is a naturally occurring precursor to BH4 which is a cofactor for nitric oxide synthetase, caution is recommended during concomitant use of sapropterin dihydrochloride with all medicinal products that cause vasodilation, including those administered topically, by affecting nitric oxide (NO) metabolism or action including classical NO donors (e.g. glyceryl trinitrate (GTN), isosorbide dinitrate (ISDN), sodium nitroprusside (SNP), molsidomin), phosphodiesterase type 5 (PDE-5) inhibitors and minoxidil. Therefore, the risk of adverse event related the risk of PD interaction, i.e., hypotension, should be considered in the section 4.5 of the SmPC. Regarding levodopa. safety data collected from healthy volunteers are not representative. The small sample sizes of the studied populations (healthy volunteers and patients) do not allow definitive conclusions about the safety of the treatment in a larger population with regard to interactions between levodopa and sepiapterin to be drawn. While the existing data are reassuring, they do not confirm the absence of interactions. Given the mechanism of action of sepiapterin, the Applicant was asked to include the interaction with levodopa in the SmPC. The Applicant agreed to add in the SmPC the interaction with NO donors and levodopa as proposed. Additional text has been added to Section 4.5 of the SmPC and Section 2 of the Patient Information Leaflet about risks with vasodilatory drugs and levodopa.

Laboratory findings

No notable laboratory trends were noted in any of the treatment groups, similarly laboratory changes from baseline were generally unremarkable.

ADRs proposed in the SmPC

According to EC SmPC Guideline rev. 2, this section should include all adverse reactions from clinical trials, post-authorisation safety studies and spontaneous reporting for which, after thorough assessment, a causal relationship between the medicinal product and the adverse event is at least a reasonable possibility. Adverse events, without at least a suspected causal relationship, should not be listed in the SmPC.

The Applicant used the safety analysis set from the pool of study PTC923-MD-003-PKU and study PTC923 MD 004 PKU to elaborate the frequency. However, this pool includes placebo patients. This does not accurately represent the real number of subjects exposed to sepiapterin. The frequencies of ADR in SmPC were therefore not representative of the actual exposure to sepiapterin. The Applicant was asked to review these frequencies. The Applicant provided a table containing the frequencies of AEs excluding placebo participants.

Here are the updated frequencies for the ADRs, considering the 168 subjects exposed, excluding those exposed to placebo.

MedDRA SOC	**ADR (PT)**	**Frequency[a] [Crude Overall Frequency] n (%)**
Gastrointestinal disorders	Diarrhoea	22 (13.1%)
Infections and infestations	Upper respiratory tract infection	35 (20.8%)

MedDRA SOC	ADR (PT)	Frequency[a] [Crude Overall Frequency] n (%)
Nervous system disorders	Headache	25 (14.9%)
Gastrointestinal disorders	Abdominal pain	7 (4.2%)
Gastrointestinal disorders	Faeces discoloured	7 (4.2%)

Therefore, the frequency stated in the table under section 4.8 of the SmPC was not adequate. The Applicant was requested to amend as follows:

- Very common: Upper respiratory tract infection, Headache, Diarrhoea

- Common: Abdominal pain and Faeces discoloured.

For the ADR Abdominal pain, the frequency stated in table on ADR in the section 4.8 of the SmPC was requested to be amended. PTC acknowledged that the frequency of the individual PT of abdominal pain would be categorised as "common"; however, the ADR of "Abdominal pain" in the ADR table is a medical concept comprising the following 3 PTs: Abdominal pain (4.5%), Abdominal pain upper (5.0%), and Abdominal discomfort (2.7%). The frequency of the ADR "Abdominal pain" based on the combination of these 3 PTs is 12.2%, and this is therefore classified in the ADR table as "very common". The table in Section 4.8 of the SmPC was revised to clarify that this ADR includes these 3 individual PTs.

In the SmPC for sapropterin, hypophenylalaninaemia is listed as a common ADR (≥ 1/100 til < 1/10). In section 4.2 of the SmPC it is stated that blood phenylalanine levels may decrease below the desired therapeutic level, and that adjustment of the Kuvan dose or modification of dietary phenylalanine intake may be required. In addition, section 4.4 warns that prolonged exposure to low blood phenylalanine and tyrosine levels during infancy has been associated with impaired neurodevelopmental outcome. The Applicant is asked to provide an overview of the frequency of hypophenylalaninaemia reported in studies performed with sepiapterin and further discuss whether hypophenylalaninaemia should be listed as an ADR in section 4.8 in the SmPC and the need for warnings in section 4.2 and/or section 4.4. It is acknowledged that the risk of hypophenylalaninaemia in response to sepiapterin is considered to be relatively low. However, the persistent low hypophenylalaninaemia can be serious and was for that reason that the promoter decided to include a well-structured rescue therapy in the open-label extension Study PTC923-MD-004-PKU Protocol. In this study it is even proposed a discontinuation from study drug administration if the rescue therapy does not work. This risk could be especially serious in children. The Applicant updated Section 4.2 and Section 4.8 of the SmPC, respectively, to warn regarding this risk of and to include hypophenylalaninaemia as an ADR.

Overall, sepiapterin was well tolerated in subjects in PKU studies across all age groups, sexes, races, and ethnicities and during long-term treatment (exposures of up to 20 months). No deaths have been reported in any study of sepiapterin, and only 1 SAE has been reported in subjects with PKU (considered unrelated to sepiapterin). Few subjects in the PKU studies have discontinued sepiapterin treatment prior to study completion due to TEAEs.

Based on the cumulative evaluation of all relevant study data for sepiapterin, the following have been evaluated as risks with sepiapterin treatment:

- Important identified risks: *None*
- Important potential risks: *None*
- Identified risk (non-important): *Gastrointestinal effects*
- Potential risk (non-important): *Drug-drug interactions with inhibitors of DHFR (eg, trimethoprim, methotrexate, pemetrexed, pralatrexate, and trimetrexate)*

These risks apply to all PKU subpopulations, including paediatrics, with no differences in safety observed between different groups of patients treated with sepiapterin.

"Gastrointestinal effects" is considered an identified risk (non-important) based upon the frequency of such events reported with sepiapterin use. This is agreed because, in the PTC923-MD-003-PKU and study PTC923 MD 004 PKU, these effects were the most frequently reported treatment-related TEAEs by SOC. Gastrointestinal effects are not an important risk of sepiapterin as all the TEAEs observed were non-serious, with the majority being mild in severity and resolved, and can be managed in clinical practice using standard of care.

Diarrhoea is listed as a very common (≥1/10) adverse drug reaction, as it is the most reported TEAE in study PTC923-MD-003-PKU: 5.1% in part 1 with 3.9% considered related to the treatment and 7.1 % in part 2 versus 1.9% in the placebo arm. Faeces discoloured and abdominal pain listed as common (≥1/100 to <1/10) adverse reactions in the SmPC Section 4.8. In the part 1 of the study PTC923-MD-003-PKU, 2.6% subjects experienced faeces discoloured and 1.9% subjects experienced abdominal pain. It was considered related to the treatment. In the part 2, 3.6% subjects experienced faeces discoloured and 1.9 % subjects experienced abdominal pain versus 0% in the placebo arm. This TEAEs were considered related to the treatment.

In the part 1 of the study PTC923-MD-003-PKU, 1.9% subjects experienced Nausea and Vomiting. It was considered related to the treatment. The Applicant was requested to justify why these TEAE are not present in the section 4.8 of the SmPC. Of note, these effects are included in section 4.8 of the Kuvan SmPC. The Applicant provided a clarification regarding the absence of these TEAEs in section 4.8 of the SmPC. It was agreed that currently available data cannot allow a causal association to be made between the reported adverse events of nausea and vomiting with sepiapterin to be made.

Upper respiratory tract infection and Headache are mentioned in the section 4.8 of the SmPC of sepiapterin which is agreed. Indeed, in part 1 of the study PTC923-MD-003-PKU, subjects experienced Upper respiratory tract infection and headache (4.5% each). In part 2, 7.1% subjects experienced headache in sepiapterin group versus 1.9% in placebo group. This difference in frequency justifies the inclusion of Headache in section 4.8 of the SmPC of sepiapterin. In PTC923 MD 004 PKU study, 12.5% subjects experienced Upper respiratory tract infection and 11.5% experienced Headache.

The Applicant was requested to discuss the two cases of hypersensitivity reported in studies PTC923 MD 003 PKU and PTC923 MD 004 PKU. The Applicant was requested to justify why these cases were deemed unrelated to the treatment and discuss the onset timing of these events. Of note, hypersensitivity is listed as a TEAE in section 4.8 of Kuvan SmPC. The Applicant clarified that the 2 events of hypersensitivity are considered both as allergies unrelated to study treatment. Details of these two cases are provided. It is acknowledged that the details provided on the two cases of hypersensitive reaction do not allow any conclusion to be drawn on sepiapterin causality. Of note, the specific symptoms of these hypersensitivity reactions are not provided. However, considering, in general, the seriousness of hypersensitivity reactions, this AE will be carefully monitored upon forthcoming PSURs.

The safety profile of sepiapterin is favourable; there were no deaths reported or TEAEs related or possibly related to study treatment. All the secondary effects are reported in the SmPC in an explicit and clear form. The safety profile is in line with the non-clinical data and physiopathology of the mechanism of action. There are no special concerns in paediatric population or other special populations, but this is not supported by available data. The data on special populations such as renal and hepatic impaired and the elderly exposed are currently limited. The on-going study PTC923-MD-004-PKU is very important to clarify the long-term safety and the safety in patients < 2 years but in addition the Applicant was requested to specify how paediatric population and other special populations will be monitored in the future. In the response provided, the Applicant considered that routine pharmacovigilance activities in the form of ADR reporting and signal detection from all sources, including the following 4 ongoing clinical trials, will adequately monitor all special populations being treated with sepiapterin:

- PTC923HI104HV, a study of PK in participants with hepatic impairment and participants with normal hepatic function
- PTC923RI103HV, a study of PK in participants with renal impairment and participants with normal renal function
- PTC923PKU401, a Phase 3b open-label study of long-term neurocognitive outcomes with sepiapterin treatment in paediatric participants under the age of 12 years with PKU
- PTC923PKU301, a Phase 3, randomised, crossover, open-label, active-controlled study of sepiapterin versus sapropterin in participants with PKU, which includes paediatric participants aged 2 to ≤17 years.

In addition, in the EU risk management plan (RMP) for sepiapterin, the ongoing Study PTC923MD004PKU is an additional pharmacovigilance activity for characterising the missing information of long-term safety. Nevertheless, data from hepatic and renal impairment patients are still missing. It is stated that Applicant is performing dedicated renal and hepatic impairment studies have been initiated. The Applicant confirmed that dedicated studies to assess the effect of renal and hepatic impairment on the pharmacokinetics (PK) and safety of sepiapterin and the active metabolite BH_4 are still ongoing, and commits to submit the final results post-authorisation via a Type-II variation.

The final data from the on-going study PTC923-MD-004-PKU will be important to set the robustness of the results on the safety of sepiapterin namely in what concerns the claimed indication (all ages).

Additional expert consultation

Not identified the need for additional expert consultation at this stage of the assessment.

Assessment of paediatric data on clinical safety

See comment above.

2.6.10. Conclusions on the clinical safety

No important risks have emerged for sepiapterin to date from clinical studies in patients with PKU.

Sepiapterin is a natural precursor of the enzymatic co-factor BH_4, a co factor for phenylalanine hydroxylase (PAH).

Sepiapterin was studied in 3 clinical studies in PKU subjects. For the safety, the phase 3 study, randomized, double blind, placebo controlled (PTC923 MD-003-PKU) and the phase 3 (PTC923 MD 004-PKU) study open label, uncontrolled are pooled. However, in this pool of safety analysis set, 54 subjects received placebo during 6 weeks in the Part 2 of the PTC923 MD-003-PKU.

A total of 529 subjects have been exposed to sepiapterin in the completed clinical studies as of the data cutoff date of 02 September 2024. This includes 275 subjects with PKU, 10 subjects with moderate to severe diabetic gastroparesis, 8 subjects with PBD, and 236 healthy volunteers.

Manageable gastrointestinal effects are reported with sepiapterin use, with diarrhoea, faeces discoloured, and abdominal pain listed as adverse drug reactions in the SmPC Section 4.8 as frequent and often treatment related. Upper respiratory tract infection and headache were also notable, justifying their inclusion in section 4.8 of the SmPC.

Frequencies proposed by the Applicant in SmPC are based on safety pool, although including patients on placebo.

Hypophenylalaninaemia has been included as an ADR in the SmPC 4.8. A related warning was also included in Section 4.2.

Drug-drug interactions with inhibitors of DHFR (eg, trimethoprim, methotrexate, pemetrexed, pralatrexate, and trimetrexate) may occur; therefore, caution in their coadministration is required as described in SmPC sections 4.4 and 4.5.

Interpretation of data is hindered by the small number of subjects and the absence of long-term data.

This safety profile of sepiapterin that has emerged to date applies to all PKU subpopulations, including paediatrics, with no differences in safety observed between different subgroups of patients treated with sepiapterin in clinical trials.

Updated safety data from ongoing study PTC923-MD-004-PKU including an update on the exposed patients' safety was provided and confirms an acceptable safety profile. The Applicant considers that routine pharmacovigilance activities in the form of ADR reporting and signal detection from all sources, including 4 ongoing clinical trials, will adequately monitor all special populations being treated with sepiapterin. The Applicant confirms that dedicated studies to assess the effect of renal and hepatic impairment on the pharmacokinetics (PK) and safety of sepiapterin and the active metabolite BH4 are still ongoing, and commits to submit the final results via a Type-II variation post-authorisation.

2.7. Risk Management Plan

2.7.1. Safety concerns

Table 20: Summary of safety concerns (RMP version 0.3)

Summary of safety concerns	
Important identified risks	None
Important potential risks	None
Missing information	Long-term safety

Summary of safety concerns	
	Use during pregnancy and lactation

2.7.2. Pharmacovigilance plan

Table 21: Summary of pharmacovigilance plan (RMP version 0.3)

Study Status	Summary of Objectives	Safety Concerns Addressed	Milestones	Due Dates
Category 1 - Imposed mandatory additional pharmacovigilance activities that are conditions of the marketing authorisation				
None				
Category 2 – Imposed mandatory additional pharmacovigilance activities that are Specific Obligations in the context of a conditional marketing authorisation or a marketing authorisation under exceptional circumstances				
None				
Category 3 - Required additional pharmacovigilance activities				
PTC923-MD-004-PKU A Phase 3 Open-label Study of PTC923 (Sepiapterin) in Phenylketonuria. Ongoing	PTC923-MD-004-PKU is an ongoing, long-term efficacy and safety study to evaluate the long-term safety of sepiapterin in subjects with phenylketonuria (PKU) and to evaluate changes from baseline in dietary phenylalanine (Phe)/protein consumption.	Long-term use	First subject enrolled:	14 February 2022
			Interim data cut/CSR	22 September 2023 02 September 2024
			Final CSR	September 2026

2.7.3. Risk minimisation measures

Table 22: Summary Table of pharmacovigilance Activities and Risk Minimisation Activities by Safety Concern (RMP version 0.3)

Safety Concern	Risk Minimisation Measures	Pharmacovigilance Activities
Long-term use (Missing information)	Routine risk minimisation measures: *SmPC sections 4.4 and 4.8* *PL section 2 and 4* Other routine risk minimisation measures beyond the Product Information: *SmPC section 4.2 (restricted medical prescription).* *PL section 3*	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: *None* Additional pharmacovigilance activities: *Study PTC923-MD-004-PKU*
Use during pregnancy and lactation (Missing information)	Routine risk communication *SmPC sections 4.6 and 5.3* *PL section 2* *SmPC section 4.2 (treatment must be initiated and supervised by a physician in the treatment of PKU - restricted medical prescription).*	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: *Pregnancy and Lactation Form will be sent to collect further details on pregnancy or lactation* Additional pharmacovigilance activities: *None*

Abbreviations: SmPC, summary of product characteristics.

2.7.4. Conclusion

The CHMP considers that the risk management plan version 0.3 is acceptable.

The applicant is reminded that the body of the RMP and Annexes 4 and 6 (as applicable) will be published on the EMA website at the time of the EPAR publication, so considerations should be given on the retention/removal of Personal Data (PD) and identification of Commercially Confidential Information (CCI) in any updated RMP submitted throughout this procedure.

2.8. Pharmacovigilance

2.8.1. Pharmacovigilance system

The CHMP considered that the pharmacovigilance system summary submitted by the Applicant fulfils the requirements of Article 8(3) of Directive 2001/83/EC.

2.8.2. Periodic Safety Update Reports submission requirements

The requirements for submission of periodic safety update reports for this medicinal product are set out in the Annex II, Section C of the CHMP Opinion. The Applicant did not request alignment of the PSUR cycle with the international birth date (IBD). The new EURD list entry will therefore use the EBD to determine the forthcoming Data Lock Points.

2.9. Product information

2.9.1. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the Applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use.*

2.9.2. Additional monitoring

Pursuant to Article 23(1) of Regulation No (EU) 726/2004, Sephience (Sepiapterin) is included in the additional monitoring list as it contains a new active substance which, on 1 January 2011, was not contained in any medicinal product authorised in the EU.

Therefore, the summary of product characteristics and the package leaflet includes a statement that this medicinal product is subject to additional monitoring and that this will allow quick identification of new safety information. The statement is preceded by an inverted equilateral black triangle.

3. Benefit-Risk Balance

3.1. Therapeutic Context

3.1.1. Disease or condition

Sepiapterin Oral Powder 250 mg and 100 mg, intended for the treatment of hyperphenylalaninaemia in adults and paediatric patients of all ages with phenylketonuria (PKU).

PKU is a rare, serious, and devastating disease that has a significant negative impact on quality of life (QoL). The disease is an inborn error of metabolism due to autosomal recessive variants in the phenylalanine hydroxylase (PAH) gene. This enzyme converts the essential amino acid phenylalanine (Phe) into tyrosine (Tyr), the precursor of many neurotransmitters, including epinephrine, norepinephrine, and dopamine. It has tetrahydrobiopterin (BH_4) as the natural cofactor that acts as a chaperone in case of structural and conformational variants of PAH thereby stabilizing the deficient enzymes allowing the enhancement of their intrinsic residual activity. In case of uncontrolled PKU, the deficiency in PAH leads to an accumulation of Phe and consequent reduction in the levels of Tyr which combined may result in severe neurologic manifestations with potential cognitive impairment.

The disease severity is related to Phe blood levels [Classical PKU - Phe >1200 µmol/L; Mild PKU – 600 < Phe < 1200 µmol/L; Mild HPA - 120 < Phe < 600 µmol/L)

Target clinical Phe blood levels are

Patient Group	Target Range for Blood Phe Concentration
⩽12 years and women before and during pregnancy	120 to 360 µmol/L
>12 years	120 to 600 µmol/L

3.1.2. Available therapies and unmet medical need

The current therapies and management options for PKU are recognised to have significant limitations.

Lifetime dietary restriction of Phe is the first line approach and has recognized limitations in efficacy. In some patients, even if strict diet is followed, target Phe levels are not reached. Patients that respond to diet restrictions are known to have trouble to adhere with the very stringent diet in the long-term and nutritional deficiencies, impaired quality of life and psychosocial status due to the burden of living with the dietary restrictions needed to ensure disease control usually develop.

Currently two products have been approved in the EU for the treatment of HPA in patients with PKU based on a reduction in blood Phe concentration:

- Sapropterin dihydrochloride (e.g., Kuvan) – although efficacious, in some patients, sub-optimal results are achieved, and others (mostly cPKU patients) may not respond at all; poor bioavailability limit its action.
- Pegvaliase (Palynziq) - indicated for second-line treatment of PKU in patients 16 years of age and older who have inadequate blood Phe control (blood Phe levels >600 µmol/L) despite prior management with available treatment options. The treatment is limited to adolescents older than 16 years and is associated with considerable side effects that limit patient acceptability and tolerability.

Development of new and more efficacious treatments that can be used in patients with insufficient response to sapropterin or <16 years old and thus not eligible for pegvaliase is needed.

3.1.3. Main clinical studies

The main evidence of efficacy submitted is sourced from Study PTC923-MD-003-PKU (completed), a phase 3, double-blind, placebo-controlled, randomized, multicentre efficacy study, comparing the effect on blood Phe levels of 6 weeks treatment with sepiapterin (n=49) to placebo (n=49) in patients that had shown a mean percent reduction in blood Phe levels of ≥30% during Part 1 (14 days treatment with sepiapterin).

3.2. Favourable effects

Based on its mechanism of action and the submitted data of clinical trial results, sepiapterin may represent a significant improvement of the treatment of PKU patients and fulfils some of the current needs as:

1. Sepiapterin treatment results in a significant and sustained decreases in Phe levels in patients at different levels of baseline Phe blood levels (disease severity) both in patients > 2 and <2 years old. Majority of patients achieved Phe blood levels below the clinically agreed "controlled disease" threshold and a significant number of patients reached "normal" Phe levels.
2. Sepiapterin treatment allowed for a higher Phe dietary consumption with beneficial impact on patient's quality of life (QoL) – based on preliminary data from an ongoing study. An update of the data was provided after the initial submission of this ongoing study and demonstrated consistently, in the different domains that were assessed, the benefit of the treatment with sepiapterin.
3. Supportive data of higher efficacy over sapropterin was generated
    a. Sepiapterin was efficacious in patients not responding to sapropterin

b. Sepiapterin improved clinical response in those patients responding to sapropterin

c. Sepiapterin seemed superior to sapropterin when analysing comparative efficacy results (larger responder rate and higher proportion of patients achieving the target Phe levels)

4. Sepiapterin offers an alternative to classic PKU patients <16 years of age (not eligible to be treated with pegvaliase) and whom have insufficient response to sapropterin.

3.3. Uncertainties and limitations about favourable effects

At submission, < 2 years old data initially submitted in support of this application was found to be insufficient to justify an indication in this population.

In its responses submitted to the D120 list of question, the Applicant has submitted an update to the preliminary results (cut-off date 02 September 2024) from an ongoing open label study evaluating the long-term safety of sepiapterin as well as the impact of sepiapterin treatment in changes in dietary Phe/protein consumption (study PTC923-MD-004-PKU). The data provided to date are based on 14 participants <2 years of age. However, results of the Dietary Phe Tolerance Assessment at week 26 (primary efficacy endpoint) were only available for 4 patients, which is low even for a rare disease. Following a new request for updated data (cut-off 13 February 2025), the Applicant updated the number of patients < 2 years of age to 33 (previously 15), 8 aged <1 year. Of the 33 patients, 8 completed the 26 weeks Dietary Phe Tolerance Assessment, with 7 other still ongoing.

Blood Phe levels were assessed in the randomised double-blind, placebo-controlled part 2 of the pivotal study (study 003), that only included subjects who previously showed a blood Phe level reduction under treatment with sepiapterin in the part 1 of the study. Section 4.2 of the SmPC was updated to include information regarding lack of controlled efficacy and safety data in patients who do not experience a reduction of 15% or greater reduction in blood Phe levels after receiving sepiapterin for 14 days.

No data has been generated on sepiapterin use during pregnancy and lactation. The received Patient Association input spotted a high unmet need in this specific population that has a high catabolic status and in which severe diet restriction is more challenging as it might have an impact on the mother nutritional status and foetus development. Also, a tight Phe blood level control is crucial to reduce foetus exposure to high Phe levels and insure normal development.

3.4. Unfavourable effects

Sepiapterin was well tolerated in subjects in PKU studies across all age groups, sexes, races, and ethnicities and during long-term treatment (exposures of up to 20 months). No deaths have been reported in any study of sepiapterin, and only 1 SAE has been reported in subjects with PKU (considered unrelated to sepiapterin). Only a few subjects in the PKU studies have discontinued sepiapterin treatment prior to study completion due to TEAEs.

Based on the cumulative evaluation of all relevant study data for sepiapterin, the identified unfavourable effects are essentially of gastrointestinal nature.

These effects are considered non-important based upon the severity and frequency of such events reported with sepiapterin use: Diarrhoea is listed as a very common (≥1/10) while faeces discoloured, and abdominal pain are listed as common (≥1/100 to <1/10).

Gastrointestinal effects are not an important risk of sepiapterin as all the TEAEs observed were non-serious, with the majority being mild in severity and resolved, and can be managed in clinical practice using standard of care.

3.5. Uncertainties and limitations about unfavourable effects

Sepiapterin's safety profile is favourable, without any deaths or SAE connected with the drug.

The sample size and study design are adequate, and the clinical findings are in line with the pre-clinical results without any relevant safety problems.

Information on potential hypophenylalaninaemia is included in the SmPC (sections 4.2 and 4.8).

No data on the use in patients with renal and/or hepatic impairment has been provided. Dedicated studies have been initiated and the Applicant has committed to submit the final results post-authorisation, upon availability, via a Type-II variation.

In the context of the supportive Study PTC923-MD-004-PKU which is ongoing, further evidence is expected to be generated about long term effects and safety in patients (mainly under the age of two years) as well as, pregnant and breastfeeding women. The data currently available, after the new requested update, is still considered insufficient to conclude on the long-term effects and safety in special populations (pregnancy and breastfeeding). Additional study data from the ongoing clinical study PTC923-MD-004-PKU will provide more insight into the long-term clinical safety of sepiapterin. It is unknown whether these data are in line with the submitted clinical safety data.

3.6. Effects Table

Table 23. Effects Table for [Sepiapterin, hyperphenylalaninaemia (HPA) in adult and paediatric patients with phenylketonuria (PKU)] (data cut-off: Study PKU-002 - 24 July 2019; Study PTC923 MD-003-PKU - 03 April 2023; Study PTC923 MD-004-PKU – 02 September 2024).

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
Favourable Effects						
Phe Blood decrease	Actual change from baseline in blood Phe concentration (mean). Phe blood levels are a biochemical surrogate of clinical control.	µmol /L	-210.1 (60mg/kg/d) -143.7 (20mg/kg/d)	-90.8 (sapropterin 20mg/Kg /d)		Study PKU-002

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
Phe Blood decrease at the end of the randomised placebo-controlled (RPC) part of the trial	Mean change at Weeks 5 and 6 from baseline (µmol/L) (primary analysis set with Phe reduction from baseline ≥ 30% during Part 1) Phe blood levels are a biochemical surrogate of clinical control.	µmol /L	-410.1	-16.2	No placebo-controlled data in <2y	Study PTC923-MD-003-PKU
Increase in mean daily Phe consumption	Mean daily Phe consumption while maintaining Phe levels < 360 µmol/L	mg/kg/day	62.5 (at Week 26)	27.6 (at baseline)	Interim data	Study PTC923-MD-004-PKU
Unfavourable Effects						
Diarrhoea	Incidence and severity of diarrhoea	%	6.4% mild diarrhoea in the initial 12 weeks of treatment. Majority resolved in short term, 2/19 where recovering		None needed special treatment	Study PTC923-MD-003-PKU and 004-PKU
Faeces discoloured	Incidence and severity of faeces discoloured	%	3.8% not serious faeces discoloured in the initial 12 weeks. Majority recovered.		None needed special treatment	Study PTC923-MD-002-PKU and 003-PKU
Nausea	Incidence and severity of nausea	%	4.5% mild nausea (grade 1) 1 did not recover		None needed special treatment	Study PTC923-MD-002-PKU and 003-PKU

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
Abdominal pain and Abdominal pain upper	Incidence, type and grade of abdominal pain	%	4.5% with abdominal pain grade 1 or grade 2 3.8% with abdominal pain upper all grade 1 Majority recovered during treatment			Study PTC923-MD-002-PKU and 003-PKU

Abbreviations: BH_4 – Tetrahydrobiopterin; PKU – Phenylketonuria; cPKU – Classical PKU; Phe – Phenylalanine
Notes: No favourable results from study Study PTC923-MD-004-PKU are included as only preliminary results are available at this point.

3.7. Benefit-risk assessment and discussion

3.7.1. Importance of favourable and unfavourable effects

Sepiapterin offers an alternative to pegvaliase in patients <16 years of age and in patients who have insufficient response to sapropterin.

Data from the pivotal study PTC923-MD-003-PKU showed a significant reduction in blood Phe levels after 6 weeks versus placebo in subjects aged over 2 years who responded to sepiapterin during the 2-week treatment period of part 1. In the pivotal study subjects who were documented as sapropterin hydrochloride-non-responsive at study entry responded to sepiapterin achieving a ≥30% reduction blood Phe level.

Results from the ongoing open label study PTC923-MD-004-PKU that aimed to assess long term safety, dietary Phe tolerance, QoL scores and long-term effect on blood Phe levels are preliminary but showed considerable and consistent improvements in Dietary Phe tolerance in subjects who completed the primary evaluation, irrespective of age.

No important risks have emerged for sepiapterin and no deaths or SAE associated with use of the drug were observed in clinical trials.

Direct comparison with sapropterin demonstrated non-inferiority regarding the safety profile.

Further data needs to be gathered not only for long-term safety but also for safety of the treatment of pregnant and breastfeeding women.

3.7.2. Balance of benefits and risks

The main evidence on efficacy of sepiapterin is derived from results of 2 phase 3 studies: a 2-part phase 3 (PTC923-MD-003-PKU) randomised double-blind placebo-controlled study in subjects of all age with PKU and

the interim results of an on-going open label (PTC923-MD-004-PKU) long term safety and efficacy study in subjects who previously completed a PTC-sponsored study and naïve PKU subjects.

Data from the part 2 of PTC923-MD-003-PKU showed a significant reduction in blood Phe levels after 6 weeks versus placebo in subjects aged over 2 years who responded to sepiapterin during the 2-week treatment period of part 1.

Given that this is a chronic disease, and that the treatment may be used for a lifetime, long-term data are particularly important, especially as subjects in the pivotal study were only treated for 6 weeks (2 of which were at the proposed dose). For the overall benefit-risk assessment the effect of lowering blood phenylalanine levels on dietary Phe intake and quality of life is also important.

Preliminary results from the ongoing open label study PTC923-MD-004-PKU that aims to assess long term safety and efficacy of Sephience showed an improvement of dietary Phe tolerance irrespective of age and were supportive of a beneficial effect of the treatment.

The currently available data on the safety profile of the molecule is reassuring, with no serious adverse effects reported.

3.7.3. Additional considerations on the benefit-risk balance

Input has been received by one patients' organisation. They stress the high burden of diet restriction and the impact of great uncertainty of its efficacy by both patients and caregivers.

According with the input received the potential impact of sepiapterin is anticipated to:

a) achieve better control of Phe levels at similar diet, which in turn will lead to less detrimental consequences on neurodevelopment and mental health;

b) may help patients not previously controlled despite diet;

c) may allow to have a less restrictive diet with nutritional but mostly QoL benefits and impact on mental health;

d) anticipated benefits in women willing to become pregnant still need to be demonstrated.

e) be a valid option for older patients still alive, probably suffering from mental and physical disabilities due to late diagnose and that no longer have family backup to control diet. Sepiapterin easy administration and safety profile would be an interesting option in these special population.

Input has also been received from a Healthcare Professionals organisation who recognises that there is still an unmet need for Phe lowering treatments specially to help / allow more patients to have Phe levels in target, to alleviate the strict diet and diminish the consequences of the burden of treatment. In addition, existing treatments have limitations both in terms of efficacy and safety.

If adult patients are in need due to higher challenge for keeping a strict diet (saturation following years of compliance), children and pregnant women have a high need as controlled Phe levels are crucial to not compromise neurodevelopment but also due to nutritional consequences on growth caused by the needed diet restrictions (a diet restrictions relief is considered important).

The possible positive impact on pregnant women is highlighted by both patients and HCP. This has not been addressed in the clinical development.

3.8. Conclusions

The overall benefit/risk balance of Sephience is positive.

4. Recommendations

Similarity with authorised orphan medicinal products

The CHMP by consensus is of the opinion that Sephience is not similar to Palynziq within the meaning of Article 3 of Commission Regulation (EC) No. 847/2000. See Appendix on Similarity.

Outcome

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considers by consensus that the benefit-risk balance of Sephience is favourable in the following indication(s):

Sephience is indicated for the treatment of hyperphenylalaninaemia (HPA) in adult and paediatric patients with phenylketonuria (PKU).

The CHMP therefore recommends the granting of the marketing authorisation subject to the following conditions:

Conditions or restrictions regarding supply and use

Medicinal product subject to restricted medical prescription (see Annex I: Summary of Product Characteristics, section 4.2).

Other conditions and requirements of the marketing authorisation

- **Periodic Safety Update Reports**

The requirements for submission of periodic safety update reports for this medicinal product are set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and any subsequent updates published on the European medicines web-portal.

The marketing authorisation holder shall submit the first periodic safety update report for this product within 6 months following authorisation.

Conditions or restrictions with regard to the safe and effective use of the medicinal product

- **Risk Management Plan (RMP)**

The marketing authorisation holder (MAH) shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the marketing authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.

New Active Substance Status

Based on the CHMP review of the available data, the CHMP considers that sepiapterin is to be qualified as a new active substance in itself as it is not a constituent of a medicinal product previously authorised within the European Union.

Refer to Appendix on new active substance (NAS).