28 February 2019
EMA/CHMP/181920/2019
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Palynziq

International non-proprietary name: pegvaliase

Procedure No. EMEA/H/C/004744/0000

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

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Administrative information

Name of the medicinal product:	Palynziq
Applicant:	BioMarin International Limited Shanbally, Ringaskiddy County Cork Ireland P43 R298
Active substance:	pegvaliase
International Non-proprietary Name:	pegvaliase
Pharmaco-therapeutic group (ATC Code):	other alimentary tract and metabolism products, (A16A)
Therapeutic indication(s):	Palynziq is indicated for the treatment of patients with phenylketonuria (PKU) aged 16 years and older who have inadequate blood phenylalanine control (blood phenylalanine levels greater than 600 micromol/l) despite prior management with available treatment options.
Pharmaceutical form(s):	Solution for injection
Strength(s):	2.5 mg, 10 mg and 20 mg
Route(s) of administration:	Subcutaneous use
Packaging:	Pre-filled syringe (glass)
Package size(s):	1 pre-filled syringe and 10 pre-filled syringes

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List of abbreviations

ACTH	adrenocorticotropic hormone
ADA	anti-drug antibodies
ADHD-RS	Attention Deficit Hyperactivity Disorder Rating Scale IV
AE	adverse event
ALT	alanine aminotransferase
ANCOVA	analysis of covariance
AST	aspartate aminotransferase
AUC	Area under the concentration-time curve
AUC(0-∞)	Area under the concentration-time curve from time 0 to infinity
AUC(0-tau) ss	Area under the concentration-time curve over the dosing interval at steady-state
AvPAL	phenylalanine ammonia lyase
BE	bioequivalence
BioMarin	BioMarin Pharmaceutical Inc.
BLQ	Below the limit of quantitation
BMI	body mass index
BOCF	baseline observation carried forward
BSA	Body Surface Area
C3, C4	complement C3, C4
CCI	Container Closure Integrity
CDF	cumulative distribution function
CFR	Code of Federal Regulations
CFU	colony forming unit
CI	confidence interval
CIC	circulating immune complexes
CL	Clearance
CL/F	apparent clearance
Cmax	Maximum concentration
Cmin	Minimum concentration
CNS	central nervous system
CPA	Critical Process Attribute
CPK	creatine phosphokinase
CPP	Critical Process Parameter
CRA	clinical research associate
CrCL	Creatinine clearance
CRF	Case Report Form
CSR	Clinical Study Report
CTCAE	Common Terminology Criteria for Adverse Events
CTD	Common Technical Document
Ctrough	trough concentration
CV	Coefficient of variation
DBP	diastolic blood pressure
DMC	Data Monitoring Committee
DoE	Design of Experiments
DSM	Diagnostic and Statistical Manual of Mental Disorders
E. coli	scherichia coli
E. coli	Escherichia coli
ECG	electrocardiogram
eCRF	electronic Case Report Form
eGFR	Estimated glomerular filtration rate
ELISA	Enzyme-linked immunosorbent assay
ESR	erythrocyte sedimentation rate
F	(relative) bioavailability
FBDS	formulated bulk drug substance
FDA	Food and Drug Administration
GAD	generalized anxiety disorder
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
GPC	Gel Permeation Chromatography
HAE	hypersensitivity adverse event
HCP	Host Cell Protein
HIPAA	Health Insurance Portability and Accountability Act

HMWS	High Molecular Weight Species
HPLC	High Performance Liquid Chromatography
hsCRP	high sensitivity C-reactive protein
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
IgE	immunoglobulin E
IgG	immunoglobulin G
IgM	immunoglobulin M
IP	investigational product
IPTG	isopropyl-beta-D-thiogalactopyranoside
IRB	Institutional Review Board
ISR	Injection-site reaction
ITT	intent-to-treat
IV	intravenous
IWRS	interactive web-response system
Ka	Absorption rate constant
Kg	kilogram
L	Liter
LOCF	last observation carried forward
LOQ	lower limit of quantitation
LS	least squares
MCB	Master Cell bank
MedDRA	Medical Dictionary for Regulatory Activities
MIO	methylidene imidazolone
mm Hg	millimeter of mercury
MMRM	mixed-effect model repeated measure
MNT	medical nutrition therapy
mPEG	methoxypolyethylene glycol
mPEG-HC	mPEG-hexanoic acid
NAb	neutralizing antibodies
NHS-PEG	N-Hydroxysuccinimide-activated linear methoxypolyethylene glycol
NIAID/FAAN	National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network
NIH	National Institutes of Health
NOR	Normal Operating Range
NSAID	Non-steroidal anti-inflammatory medication
PAH	phenylalanine hydroxylase
PAL	phenylalanine ammonia lyase
PAR	Proven Acceptable Range
PD	pharmacodynamic
PEG	polyethylene glycol
PFS	prefilled syringe
Ph. Eur.	European Pharmacopoeia
Phe	phenylalanine
PK	pharmacokinetics
PK/PD	pharmacokinetic/pharmacodynamic
PKU	phenylketonuria
PKUDOS	PKU Demographics, Outcomes and Safety
PKU-POMS	phenylketonuria-specific Profile of Mood States
POMS	Profile of Mood States
Pop-PK	Population PK
PPQ	Process Performance Qualification
PT	preferred term
QbD	Quality by Design
rAvPAL	rAv phenylalanine ammonia lyase
rAvPAL-PEG	rAv phenylalanine ammonia lyase-PEG
RDA	recommended dietary allowance
RP-HPLC	Reverse Phase-High Performance Liquid Chromatography
SAE	serious adverse event
SAP	Statistical Analysis Plan
SBP	systolic blood pressure
SD	standard deviation
SE	standard error

SEC	Size-Exclusion Chromatography
SGOT	serum glutamic-oxaloacetic transaminase (see AST)
SGPT	serum glutamic-pyruvic transaminase (see ALT)
SMQ	Standardized MedDRA Queries
SOC	system organ class
SpO2	periphenylalanineral capillary oxygen saturation
SSLR	serum sickness-like reaction
TAb TAE TMD	Total anti-pegvaliase antibodies treatment-emergent adverse event Total Mood Disturbance
ULN US	upper limit of normal United States
USA	United States of America
USP	United States Pharmacopeia
V	Volume of distribution
V/F	apparent volume of distribution
V/S	vial/syringe
VS	vial and syringe
WCB	Working Cell Bank
µmol	micromol

1. Background information on the procedure

1.1. Submission of the dossier

The applicant BioMarin International Limited submitted on 6 March 2018 an application for marketing authorisation to the European Medicines Agency (EMA) for Palynziq, 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 January 2017.

Palynziq was designated as an orphan medicinal product EU/3/09/708 on 28/01/2010 in the following condition: Treatment of hyperphenylalaninaemia.

The applicant applied for the following indication:

Palynziq is indicated for the treatment of adults with phenylketonuria (PKU) who have inadequate blood phenylalanine control (blood phenylalanine levels greater than 600 µmol/) despite prior management with available treatment options including sapropterin.

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 studies.

Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision(s) P/0036/2017 on the agreement of a paediatric investigation plan (PIP).

At the time of submission of the application, the PIP P/0036/2017 was not yet completed as some measures were deferred.

Information relating to orphan market exclusivity

Following the CHMP positive opinion on this marketing authorisation, the Committee for Orphan Medicinal Products (COMP) reviewed the designation of Palynziq as an orphan medicinal product in the approved indication. More information on the COMP's review can be found in the Orphan maintenance assessment report published under the 'Assessment history' tab on the Agency's website: ema.europa.eu/Find medicine/Human medicines/European public assessment reports. (https://www.ema.europa.eu/en/search/search?search_api_views_fulltext=EU/3/09/708)

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.

New active Substance status

The applicant requested the active substance pegvaliase contained in the above medicinal product to be considered as a new active substance, as the applicant claims that it is not a constituent of a medicinal

product previously authorised within the European Union.

Protocol assistance

The applicant did not seek Protocol assistance at the CHMP.

1.2. Steps taken for the assessment of the product

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

Rapporteur: Johann Lodewijk Hillege Co-Rapporteur: Alexandre Moreau

The application was received by the EMA on	6 March 2018
The procedure started on	29 March 2018
The Rapporteur's first Assessment Report was circulated to all CHMP members on	19 June 2018
The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on	18 June 2018
The PRAC Rapporteur's first Assessment Report was circulated to all PRAC members on	2 July 2018
The CHMP agreed on the consolidated List of Questions to be sent to the applicant during the meeting on	26 July 2018
The CHMP agreed on the consolidated revised List of Questions to be sent to the applicant adopted via written procedure on:	23 August 2018
The applicant submitted the responses to the CHMP consolidated List of Questions on	12 October 2018
The following GLP inspection(s) were requested by the CHMP and their outcome taken into consideration as part of the Quality/Safety/Efficacy assessment of the product: – A GLP inspection was performed by FDA at the sponsor site in the USA between 05-09 November 2018. The outcome of the inspection carried out was issued on:	23 January 2019
The Rapporteurs circulated the Joint Assessment Report on the responses to the List of Questions to all CHMP members on	20 November 2018
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	29 November 2018
The CHMP agreed on a list of outstanding issues in writing and/or in an oral explanation to be sent to the applicant on	13 December 2018
The applicant submitted the responses to the CHMP List of Outstanding Issues on	28 January 2019
The Rapporteurs circulated the Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP members on	14 February 2019

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 Palynziq on	28 February 2019
The CHMP adopted a report on similarity of Palynziq with name of the authorised orphan medicinal product(s) on (Appendix 1)	28 February 2019

2. Scientific discussion

2.1. Problem statement

Phenylketonuria (PKU) is an inherited, autosomal recessive disease characterized by a deficiency in the intracellular liver enzyme phenylalanine hydroxylase (PAH). PAH catalyses the conversion of the essential amino acid phenylalanine to tyrosine, and this enzymatic activity is facilitated by tetrahydrobiopterin (BH4). PAH deficiency results in an abnormally elevated concentrations of phenylalanine, which is toxic to the brain (Kaufman, 1989).

High phenylalanine levels during infancy and early childhood cause profound cognitive and developmental defects, and poorly controlled blood phenylalanine levels in older children and adolescents are associated with learning disabilities, attention deficit hyperactivity disorder, and behavioural problems (Moyle, 2007; Moyle, 2007; Pietz, 1997; Smith, 2000; Waisbren, 1999; Waisbren, 2007; Stemerdink, 2000). With new-born screening followed by dietary phenylalanine-restriction, the most severe manifestation of the disease — mental retardation — is prevented.

However, most adults with PKU do not adhere to the dietary phenylalanine restriction to control blood phenylalanine levels; 79% of adolescents and 78% of adults with PKU have blood phenylalanine levels above the recommended target range of 60-600 µmol/L (Waisbren, 2007; Walter, 2002). Uncontrolled blood phenylalanine levels in adulthood are associated with executive dysfunction, and a variety of behavioural and psychiatric problems (Moyle, 2007; Pietz, 1997; Smith, 2000; Waisbren, 1999).

Adults with phenylketonuria (PKU) may experience neurologic and psychiatric disorders, including intellectual disability, anxiety, depression, and neurocognitive dysfunction. For most of the adult patients this will impact the quality of life, especially as they encounter problems in social interactions. In contrast, there are also adult PKU patients who do not have their blood phenylalanine levels checked regularly or even are lost on follow-up.

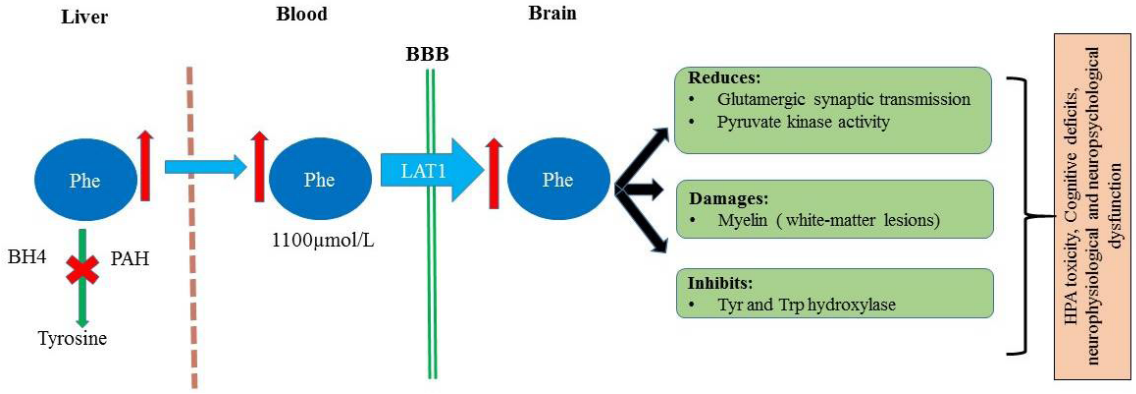
2.1.1. Epidemiology

The prevalence of PKU shows considerable geographic variation. The estimated prevalence of PKU in adults in the European Community is 1:10,000 (van Spronsen, 2017), ranging from 0.1:10,000 in Finland to 2.2:10,000 in Slovenia (Loeber, 2007; Guldberg, 1995; Van Spronsen, 2017). The prevalence of PKU in the United States is estimated to be 0.67:10,000 (NIHCDP, 2001). As a result of newborn screening efforts implemented in the 1960s and early 1970s, virtually all individuals with PKU under the age of 40 are diagnosed at birth and treatment is implemented soon thereafter. In Northern Europe, there is a relative large proportion (around 60%) of patients with the severe form of PKU. In Southern Europe this proportion is around 40%. Patients with this more severe form of PKU do not have residual PAH enzyme activity and do not respond to sapropterin treatment, the synthetic cofactor for the PAH enzyme.

2.1.2. Aetiology and pathogenesis

PAH is most abundant in the liver cells and catalyses the hydroxylation of phenylalanine to tyrosine (Tyr) in the presence of the cofactor BH4. Absent or non-functional PAH activity prevents or significantly reduces the metabolism of phenylalanine to Tyr. As a result, concentrations of phenylalanine build up in the liver and in the blood, causing hyperphenylalaninaemia (HPA). Phenylalanine is also able to cross the blood-brain barrier via L-type amino acid carriers, which is the principal cause of HPA toxicity. These physiological mechanisms are summarized visually in **Figure 1**.

Figure 1. Pathophysiology of Phenylketonuria



BBB, blood-brain-barrier; BH4, tetrahydrobiopterin; LAT1, L-type amino acid transporter; PAH, phenylalanine hydroxylase; Phe, phenylalanine; Tyr, tyrosine; Trp, tryptophan.

Source: adapted from van Spronsen, 2017.

The principal consequences of HPA toxicity are neurological and psychiatric pathology and psychological and neurocognitive dysfunction (Moyle, 2007a; Moyle, 2007b, Pietz, 1997; Smith, 2000; Waisbren, 1999; Gassio, 2003). HPA and prolonged medical nutrition therapy (MNT) can also result in nutritional deficits, dysfunctional metabolic control, and bone pathology (Enns, 2010).

2.1.3. Clinical presentation, diagnosis

In patients with PKU, HPA toxicity is principally caused by elevated phenylalanine levels in the brain which is toxic to the central nervous system (CNS) and can, especially in children, result in neurological and psychiatric pathology and psychological and neurocognitive dysfunction (Bilder, 2016; Bilder, 2017; NPKUA, 2014):

- Neurological pathology: seizures, tremor, migraine, and probably long-term neurodegeneration
- Psychiatric: depression, anxiety
- Psychological: mood, inattention, impulsivity, confusion, anger, vitality, tiredness, agoraphobia
- Neurocognitive: executive function, verbal memory, inhibitory control, working memory, cognitive flexibility
    - Executive function includes planning, problem solving, information processing, bringing previous experience to bear on activities, and sustained attention

The prevalence of psychiatric and neurologic symptoms in adults with PKU is higher than the US National Institutes of Health (NIH) estimates for disease prevalence in adults in the general population (NIMH, 2013). Specifically, 49% of adults with PKU reported inattention symptoms versus 4% of adults in the general population who reported ADHD; 22% of adults with PKU reported anxiety symptoms versus 3% of adults in the general population who reported generalized anxiety disorder; and 18% of adults with

PKU reported depression symptoms versus 7% of adults in the general population who reported major depressive disorder.

Evidence from a systematic literature review and meta-analysis found that in adults executive functioning impairment impacts daily life by interfering with the ability to perform basic cognitive tasks such as focusing, memory, planning, and impulse control. These tasks play a critical role in fulfilling responsibilities of adulthood such as acquiring/maintaining employment, managing money, raising a family, and driving (Bilder, 2016). These findings suggest that neuropsychiatric and executive functioning deficits are reversible in adults with PKU. The mental state of patients is influenced by their executive function, which in turn impacts their ability to plan, organize, and follow the severe phenylalanine restriction + MNT, and their overall quality of life. Higher lifetime rather than concurrent phenylalanine-levels were related to poorer social outcomes. The influence of phenylalanine during childhood and early adolescence seems to be important for social cognitive functioning and social skills later in life (Jahja, 2016). Interestingly, average phenylalanine levels have been reported to be significantly correlated with scores for psychoticism and psychiatric symptom intensity, while better phenylalanine control during childhood was associated with better mood during adulthood. Similarly, depression and anxiety scores were positively correlated with the age at diet initiation and childhood phenylalanine levels, respectively. Equally, in other studies, psychiatric disturbance, anxiety and depression were neither associated with current dietary status, nor with concurrent or historical phenylalanine levels (Burlina, 2019).

In addition, HPA and prolonged MNT can also result in nutritional deficits (Enns, 2010), dysfunctional metabolic control, and bone pathology (Hoeks, 2009; Camp, 2014; Hvas, 2006; Schulz, 1995; Sarkissian, 2009) and increased diabetic and cardiovascular risk factors (e.g., increased lipids, homocysteine, and body mass index [BMI]) (Moseley, 2002; Macleod, 2010).

2.1.4. Management

The aim of PKU management is to identify patients at birth, to control their HPA, and to retain neurological health (IQ) and neurocognitive function as adults. Although various country-specific guidelines have been developed over the last few years, the first consensus pan-European PKU guidelines were published in 2017 (van Spronsen, 2017; van Wegberg, 2017). These guidelines for the diagnosis and treatment of PKU were developed by 19 medical specialists in the field, throughout Europe, under the auspices of the European Society for phenylketonuria and Allied Disorders (ESPKU).

There are two recommendations pertinent to adults:

- Lifelong treatment is recommended for any patient with PKU whose blood phenylalanine levels are greater than 600 µmol/L; all adults with PKU should have lifelong, systematic follow up in specialized metabolic centres due to specific risks which may occur during adulthood.
- In treated PKU patients aged >12 years (non-pregnant), the target blood phenylalanine levels are 120 to 600 µmol/L.

Phenylalanine is one of 8 essential amino acids that cannot be synthesized de novo in the human body. Thus, in the general population, physiological requirements for phenylalanine are met exclusively by dietary protein intake. Therefore, patients with PKU must primarily control their blood phenylalanine levels by controlling their dietary phenylalanine intake through the following methods, referred to as MNT:

- Severe natural protein restriction according to individual phenylalanine tolerance
- Low protein phenylalanine-free synthetic food to meet energy requirements

- Phenylalanine-free L-amino acid supplements (usually with added vitamins and minerals) to meet protein and non-protein requirements

Current treatment strategies available in Europe for PKU patients include:

- MNT in conjunction with treatment with sapropterin.

Phenylalanine concentration categories considered normal, requiring treatment, and targets for the management of treated patients with PKU in Europe are presented in **Table 1**.

Table 1. Phenylalanine Blood Concentration Categories for Disease Management

Phenylalanine Blood Concentration Category	**Phenylalanine Concentration Range (µmol/L)**
Low	<60
Normal	60-120
Target in treated patients with PKU (>12 years and non-pregnant adults)	120-600
Uncontrolled, in need of treatment	>600

Source: van Spronsen, 2017

An online survey, completed by 81 healthcare providers (HCPs) across 24 countries, carried out on behalf of the European PKU group, indicated that only 28% of adults with PKU were currently achieving phenylalanine control of less 600 µmol/L (the number of HCPs who responded to this question was 43/81) (Trefz, 2015). In a PKU systematic review and meta-analysis, even amongst those adults who received early treatment with continuous MNT (defined in most included publications as initiating a low-phenylalanine diet within 90 days of birth), mean blood phenylalanine levels ranged from 709 to 1053 µmol/L, indicating that there are significant challenges associated with the use of MNT over the long term.

Patients with the more severe form of PKU, with untreated blood phenylalanine levels of >1200 µmol/L, are unlikely to achieve control with the current available treatment options; according to the online survey from 81 HCPs in the 24 European countries, this comprises 66% of adults with "classical" PKU (Trefz, 2015). While the current EU upper threshold for phenylalanine control is 600 µmol/L, this upper threshold may be reduced, as more evidence emerges regarding the clinical benefit of the reduction in blood phenylalanine levels (e.g., the 2014 American College of Molecular Genetics and Genomics [ACMG] guidelines recommend life-long management of blood phenylalanine levels under 360 µmol/L) (Vockley, 2014).

About the product

Pegvaliase is a PEGylated recombinant phenylalanine ammonia lyase (rAvPAL), derived from the Cyanobacterium *Anabaena variabilis* expressed in *E coli*. The purpose of the PEGylation of rAvPAL is to reduce immune recognition as rAvPAL is a bacterial protein and to increase the half-life.

Pegvaliase converts phenylalanine to ammonia and trans-cinnamic acid that are metabolized by the liver and excreted in the urine, respectively. Pegvaliase reduces blood phenylalanine levels and as such substitutes for the deficient PAH enzyme. Contrary to PAH which act intracellular and is modulated by cofactor tetrahydrobiopterin (BH4) pegvaliase acts in the blood compartment independent from BH4.

The claimed indication for Palynziq was for the treatment of adults with phenylketonuria (PKU) who have inadequate blood phenylalanine control (blood phenylalanine levels greater than 600 µmol/l) despite prior management with available treatment options including sapropterin.

The indication approved by the CHMP was for the treatment of patients with phenylketonuria (PKU) aged 16 years and older who have inadequate blood phenylalanine control (blood phenylalanine levels greater than 600 µmol/l l) despite prior management with available treatment options.

The recommended starting dosage of pegvaliase is 2.5 mg administered once per week for 4 weeks. The dosage should be escalated in a step wise manner based on acceptable tolerability to reach appropriate maintenance dosage once daily.

The maintenance dosage is the lowest effective dosage to achieve and maintain blood phenylalanine control. After a minimum of 24 weeks on 20 mg/day, the dosage may be further adjusted (up titration) up to 40 mg/day.

Type of Application and aspects on development

The applicant did not request scientific advice in the EU. The only other authorised medical treatment for PKU is sapropterin (Kuvan) for which the MAH is also BioMarin. Prior to the approval of sapropterin, the applicant had received scientific advice in which blood phenylalanine levels were accepted as a surrogate endpoint for the treatment of PKU patients. The same endpoint was used during the clinical development of pegvaliase.

2.2. Quality aspects

2.2.1. Introduction

The finished product is presented as a solution for injection in pre-filled syringe containing 2.5 mg, 10 mg or 20 mg of pegvaliase as active substance. Pegvaliase is a PEGylated recombinant phenylalanine ammonia lyase (rAvPAL), derived from the cyanobacterium *Anabaena variabilis* expressed in *Escherichia coli* (*E. coli*). During manufacture, the rAvPAL protein is purified and subsequently PEGylated with 20 kDa linear NHS methoxypolyethylene glycol (NHS-PEG), forming the active substance, pegvaliase (rAvPAL-PEG). The purpose of the PEGylation of rAvPAL is to reduce immune recognition as rAvPAL is a bacterial protein and to increase the protein's half-life.

Other ingredients are: trometamol, trometamol hydrochloride, sodium chloride, trans-cinnamic acid and water for injections.

The product is available in 1 mL pre-filled syringe made of Type I borosilicate glass, equipped with a stainless steel 26 gauge needle, needle safety device, polypropylene plunger rod, and chlorobutyl rubber syringe stopper with fluoropolymer coating. The automatic needle guard is composed of a polycarbonate transparent needle guard and a stainless steel spring inside the needle guard. After injection, the spring expands causing the needle to be covered by the needle guard.

2.2.2. Active Substance

General information

Pegvaliase is an enzyme substitution therapy which works by converting phenylalanine (Phe) to trans–cinnamic acid and ammonia. Enzyme activity is attributed to the electrophilic prosthetic 4-methylideneimidazole–5–one (MIO) group.

The rAvPAL protein is a homotetramer containing 567 amino acid residues and has a molecular weight of 62 kDa per monomer. Two point mutations, converting cysteine residues into serine residues, have been introduced into the native AvPAL sequence to prevent aggregation via intramolecular disulfide formation. After translation, the residues Ala 167, Ser 168, and Gly 169 undergo autocatalytic dehydration and cyclization in order to form an electrophilic prosthetic 4-methylideneimidazole–5–one (MIO) group, which is essential for rAvPAL enzyme activity. PEGylation refers to the conjugation of the exposed lysines of the

rAvPAL protein with linear ~20 kDa methoxypolyethylene glycol (mPEG) that has been activated with an N-hydroxysuccinimide active ester (NHS–PEG).

The rAvPAL homotetramer contains 72 lysine (K) groups (18 per monomer), of which approximately 48 (12 per monomer) have some level of surface exposure for the lysine ε-amino group by which the PEG is linked. The anticipated extent of PEGylation is approximately 8-9 PEGs per monomer. The total molecular weight of pegvaliase is ~1,000 kDa.

Manufacture, characterisation and process controls

Description of manufacturing process and process controls

The active substance manufacturing process has been adequately described.

rAvPAL protein is produced in recombinant *Escherichia coli* cells. Cells from the working cell bank or master cell bank are expanded in shake flasks followed by a seed fermenter and a production fermenter using a batch-fed process. At the end of the fermentation the cells are harvested by centrifugation and the resulting cell paste can be either forward processed or frozen and stored.

The cell paste is re-suspended and homogenized to lyse the cells. The lysate is heated, diluted and clarified by centrifugation and multi-stage filtration. The clarified lysate is purified and the bulk rAvPAL is frozen in bags for long-term storage.

NHS-PEG is filtered, purified by recrystallization and dried to yield pure NHS-PEG. rAvPAL is then used as a starting material for the next step, which involves the PEGylation of rAvPAL with NHS-PEG.

The bulk rAvPAL is pooled and subsequently diafiltered. The pegvaliase FBDS is stored in single-use 10 L bags that comply with Ph. Eur. requirements and are received from the supplier sterilized and ready for use. The FBDS storage container is a multi-layered bag consisting of polyester elastomer coextruded with an ethyl vinyl alcohol barrier layer, and an ultra-low density polyethylene product contact layer. An evaluation of materials was performed to assess the leachables/extractables profile. A summary of the risk assessment for the container closure system is provided and it was found acceptable.

Pegvaliase FBDS lots (20 mg/mL) may be diluted to 5 mg/mL, as needed, for the production of the 2.5 mg / 0.5 mL syringe finished product presentation through the addition of formulation buffer. After diluting, the material is mixed and 0.2 µm filtered into the FBDS storage bag. The diluted FBDS may be processed further or stored.

The description of the manufacturing process is sufficiently detailed. Pooling strategy and manufacturing scale have been appropriately indicated. Target values and proven acceptable ranges are tabulated for critical and key operating parameters. In-process controls (IPCs) have been defined to ensure appropriate performance of the manufacturing process.

Control of materials

The construction and preparation of the expression plasmid and generation of the production cell line is described in sufficient detail. The rAvPAL expression vector was created by conventional molecular cloning techniques.

The Master Cell Bank (MCB) and one Working Cell Bank (WCB) were characterized in accordance with ICH Q5B and ICH Q5D guidance. The tests performed and results are briefly described in test reports. Adequate protocols for stability monitoring of the MCB and WCB and establishment and control of future WCBs have been provided.

The composition of media used during seed fermenter operations and production as well as the composition of buffers are adequately described. Specifications for non-compendial ingredients were

provided. No materials of human or animal origin are used in the manufacture of pegvaliase. Resins are appropriately approved before use.

In the initial application NHS-PEG was defined as a starting material (raw material), which was not considered appropriate during CHMP review. NHS-PEG was re-classified as an intermediate in the manufacturing process and appropriate quality controls were established. Information to support this classification change was provided and considered acceptable.

Control of critical steps and intermediates

The control strategy for pegvaliase FBDS is implemented by means of specifications, manufacturing process controls and an in-process testing plan. The rationale for designating critical process parameters (CPPs) as well as in-process controls is briefly discussed in this section.

The applicant has used a combination of traditional and enhanced approaches to process development. Process development and the creation of a control system strategy were structured around critical quality attributes (CQAs) of rAvPAL intermediate and pegvaliase FBDS. CPPs are defined as a process parameter whose variability has an impact on a critical quality attribute and therefore should be monitored or controlled while a critical process attributes (CPA) is a direct measure or indicator of the performance for a critical step as it relates to a process intermediate or product quality. The rationale for assigning a process parameter or attribute as critical is clearly described.

An overview of all CPP/CPA with the Proven Acceptable Range (PAR) and Normal Operating Ranges (NOR) for each parameter/attribute is provided along with a summary of the supporting data used to justify these ranges. For most CPPs/CPAs the PARs are identical to the NORs and these ranges are supported by min/max data of commercial scale batches and sometimes by small scale studies as well. For a limited number of CPP/CPA the PARs are based on small scale studies only. Reports show that these PARs are adequately substantiated.

N-Hydroxysuccinimide-activated linear methoxyPEG (mPEG-NHS or NHS-PEG) reagent which has a hexanoic acid-derived linker is an intermediate in the production of pegvaliase. The chemical structure of NHS-PEG used in the PEGylation reaction is shown **Figure 2**.

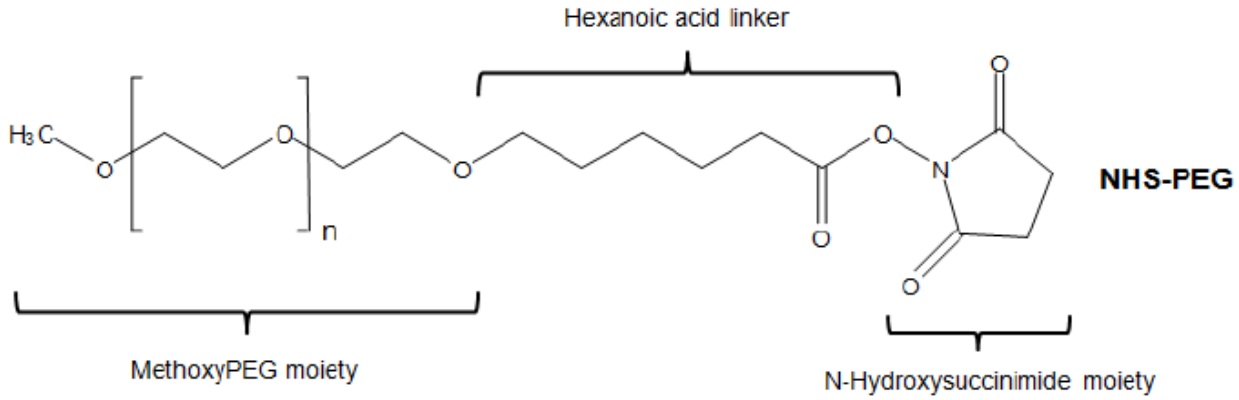


Figure 2: Chemical structure of NHS-PEG

Recombinant phenylalanine ammonia lyase (rAvPAL) is defined as a second intermediate.

Analytical methods used for the control of the rAvPAL intermediate have been described. Both a concise overview and comprehensive SOP for each method have been provided. The test methods are appropriately validated and suited for their purpose.

Process validation

The performance qualification of the commercial manufacturing process was performed by successfully manufacturing three formulated bulk active substance batches using normal processing parameter set points and conditions. The limit of *in vitro* cell age (LCA) was demonstrated by manufacturing data. Results are compiled in detailed PPQ reports and substantiate that the full scale commercial process performs effectively and is able to produce an active substance meeting its predetermined controls and acceptance criteria.

Process validation was completed and the applicant presented the in-process testing results and FBDS release testing results. These results demonstrate the manufacturing process for pegvaliase is controlled and validated, resulting in FBDS that meets in-process and release testing criteria. Comparability of the modified process with the previous process has been sufficiently demonstrated.

The lifetime of the resins used in the chromatographic purification operations was properly validated at laboratory scale. The provided data are satisfactory and support the proposed lifetimes. Clear protocols for confirmation of the proposed lifetimes at production scale are provided.

Hold times of process intermediates are defined and validation data were submitted. Proposed hold times are mainly based on historical large scale data.

Adequate validation data are provided for reprocessing, impurity clearance and shipping. The pegvaliase active substance manufacturing process has been validated adequately.

Manufacturing process development

The commercial active substance manufacturing process was developed in parallel with the clinical development program.

Over the course of clinical development, the manufacturing process was optimized in order to increase purity and yields, streamline manufacturing operations, optimize utilization of critical raw materials and control critical quality attributes for rAvPAL enzyme (a critical intermediate) and pegvaliase FBDS.

The comparability assessment assessed via batch analysis (release testing), stability analysis, and additional characterization studies for product manufactured throughout development was found acceptable.

The material produced using the proposed commercial manufacturing was used to support the entire Phase III clinical trial.

Characterisation

The rAvPAL protein intermediate was part of pegvaliase FBDS characterization analysis. This is because pegvaliase is a highly PEGylated protein, consisting of more PEG than protein on a mass basis. Furthermore, PEG is also polydisperse, i.e., yielding a variety of PEG masses attached to each PEGylation site. These features of pegvaliase make it difficult to characterize the PEGylated pegvaliase protein structure.

Most of the characterization studies on rAvPAL intermediate were performed on the reference preparation, clinical batches and 3 PPQ batches.

The primary structure of rAvPAL was confirmed by molecular mass spectrometry, N terminal sequencing, C-terminal sequencing and peptide mapping with LC-MS detection. Peptide profiles were generated on a RP-HPLC system and the active site peptide was detected using ESI-TOF-MS.

Disulfide/free-sulfhydryl determination was conducted on rAvPAL using RP-HPLC analysis to analyse the primary structure of the rAvPAL monomer.

Heterogeneity of rAvPAL was studied by a range of methods.

Size variants have been analyzed by SDS-CGE, SDS-PAGE, Western blots, SEC and dynamic light scattering.

Peptide mapping was performed using mass spectrometry.

Biological activity was adequately addressed by measuring the specific activity, kcat and Km test methods.

Almost all characterization studies on pegvaliase active substance were performed on the reference preparation, three clinical batches and three PPQ batches.

A SEC-MALS system was used to verify the molecular weights and extent of PEGylation of the pegvaliase lots.

Subtractive tryptic mapping assays with UV detection or MS detection were used to identify PEGylated lysine residues and the degree of their PEG occupancies.

Pegvaliase Dimers and multimers were studied by SEC-HPLC.

Cryo TEM and SEC-MALS in non-denaturing and denaturing conditions were used to characterise the tertiary and quaternary structure of the pegylated protein.

The pegvaliase structure was further characterized by viscosity measurements, circular dichroism, nano differential scanning calorimetry and the enzymatic assay.

Consistent results were obtained between the batches included in these studies for the quality attributes studied.

Process related impurities (Host Cell Protein (HCP) and DNA, endotoxin, antifoam, isopropyl-beta-D-thiogalactopyroanoside, kanamycin, polysorbate 80, free pegvaliase, free PEG, free N-Hydroxysuccinimide, ammonium sulfate and other PEG-related impurities) and product related impurities were appropriately discussed and evaluated.

Specification

The active substance specification includes all the critical quality attributes that affect the manufacturing and quality of the finished product. Quality Control tests for batch release include: safety (bacterial endotoxin and bioburden), identity, potency (specific activity), purity and impurities and several other general tests.

Formulated bulk active substance specification has been revised during the procedure. Clear graphical presentations are presented to support justification of acceptance criteria. The applicant commits to re-evaluate specifications based on experience gathered with further commercial lots and to amend the limits when appropriate.

Analytical methods

The analytical methods used have been adequately described and non-compendial methods appropriately validated in accordance with ICH guidelines.

Batch analysis

Batch analysis data 75 batches of development and commercial batches of the active substance were provided. The results are within the specifications and confirm consistency of the manufacturing process.

Reference materials

Qualification data for the reference materials (rAvPAL intermediate and pegvaliase) used to date, including results of release tests as well as characterisation data, were submitted. Preparation and qualification of future reference materials is sufficiently described. The reference stability protocols are adequate and stability data for the current reference materials demonstrate that these reference preparations are stable for the observed period.

Stability

The stability results indicate that the active substance is sufficiently stable and justify the proposed shelf life in the proposed container.

Long-term and accelerated stability studies have been conducted to qualify the expiry period for rAvPAL intermediate. The rAvPAL lots included in the stability assessment include a PPQ batch and subsequent commercial process batches. For the long term storage condition, no significant trends were observed. Stability studies have been completed and support the proposed holding time of the rAvPAL Intermediate.

In accordance with ICH Q5C, formal stability studies for pegvaliase FBDS were conducted on 15 batches including diluted and undiluted FBDS batches at recommended long term and accelerated storage conditions. The proposed storage time of pegvaliase FBDS is a cumulative 12 month expiry at the recommended long term storage condition.

At the recommended storage condition no trends were observed. The available stability data support the proposed shelf-life.

2.2.3. Finished Medicinal Product

Description of the product and pharmaceutical development

Pegvaliase finished product is supplied as a sterile, preservative-free solution contained in single use, glass prefilled syringe (PFS) with staked needle for subcutaneous injection.

The three different strength presentations are provided to support the dosing regimen of pegvaliase, which includes induction, titration, and maintenance (I/T/M) stages to achieve the required therapeutic dose.

The primary container closure consists of a 1 mL long Type I borosilicate glass prefilled syringe with a stainless steel staked needle. The closure consists of a stopper and a rigid needle shield. The syringe is equipped with functional secondary packaging components including the plunger rod and needle safety device. Each syringe is packaged in a heat-sealed tray and an outer carton.

Each PFS contains a slight overfill amount to ensure the correct volume is delivered upon injection.

The formulation of the finished product is the same as the active substance. Compatibility is demonstrated through formulation development studies which aimed to evaluate various stabilizing excipients for pegvaliase at long-term storage and accelerated temperature conditions. Pegvaliase finished product is physically and chemically compatible with the selected PFS primary container closure system.

The finished product solution is rather viscous, as indicated in the SmPC.

The same formulation was used in all Phase 1 through Phase 3 clinical studies for pegvaliase. During early clinical development, pegvaliase finished product was presented in a vial container closure system. The transition to a PFS was implemented to simplify the administration for the patient, improve patient

compliance with treatment, and reduce the potential for medication errors. During the Phase III clinical studies, both finished product vials and PFS were used.

Sufficient information on the different versions of the vial and pre-filled-syringe manufacturing processes developments are provided (tables not reproduced in this AR). Differences apply to manual or automated steps, fill volumes, batch sizes and holding times.

While *trans*-cinnamic acid (t-CA) used in the finished product formulation in itself may be a new excipient it is relevant to consider that t-CA is generated '*in situ'* after addition of a well-known excipient. Thus additional requirements for novel excipients with respect to details on its manufacture, specification and stability for an excipient as added to the formulation would not be appropriate.

Manufacture of the product and process controls

The Pegvaliase finished product primary manufacturing process description consists of thawing of FBDS at the active substance manufacturing site, until preparation of the fill line, pre and sterile filtration of the active substance, 'recombination' or pooling of filtered active substance and filling into syringes followed by stoppering of the syringes and an inspection process.

A process performance qualification (PPQ) of the pegvaliase finished product primary manufacturing process was conducted. The PPQ verified that CPPs were within the defined ranges and target setpoints as defined in approved batch records and standard operating procedures. PPQ batches were subjected to routine inspection and finished product release testing including container closure testing. In addition, extra non-routine in-process samples were also collected and tested including samples to demonstrate sterility, microbial control and content uniformity throughout the primary manufacturing process.

The filling duration has been qualified through the successful completion of media fill challenges for the fill line equipment.

The manufacturing process has been validated. 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.

Product specification

The analytical specifications for the finished product are primarily based on compendial requirements and/or the specifications for pegvaliase FBDS, as the filling process is not expected to cause significant changes to safety, identity, strength, quality, purity, potency, and composition attributes of pegvaliase. Functionality specifications for the syringe are based on human factors analysis and design input requirements. The proposed acceptance criteria are presented alongside the historical ranges, averages, and standard deviations and their justification for each parameter.

Analytical methods

The analytical methods used have been adequately described and (non-compendial methods) appropriately validated in accordance with ICH guidelines.

Most of the analytical methods for pegvaliase finished product are also used for pegvaliase FBDS.

Batch analysis

Analysis results of all development and full scale commercial batches are provided (approximately 80 batches). Date of manufacture, active substance batch, strength, container manufacturing facility and disposition of the batches were indicated.

The results are within the specifications and confirm consistency of the manufacturing process.

Reference materials

Reference standards are the same as those described for the active substance controls.

Stability of the product

A 2-year shelf-life at 2 - 8 °C is proposed for the finished product. The claim is based on 24 months data on primary batches, which are considered representative for the commercial presentation.

A bracketing approach as defined in ICH Q1D was used for evaluating the stability of the four PPQ batches. The use of bracketing is justified as it is applied to identical or closely related formulations and in a situation where the same container closure system is used for all presentations and either the container size or fill volume varies while the other remains constant.

The analytical methods used to monitor pegvaliase finished product stability are the same methods used for the finished product release. Container closure integrity (CCI) testing by vacuum decay is performed in lieu of sterility testing to ensure sterility of the product during the finished product shelf-life.

For the 5 ± 3 °C storage condition, the stability batches conform to all acceptance criteria through 24 months of storage.

Protocols for studies on additional batches are supplied and do not raise any issues.

The proposed shelf-life of 2 years at 5°C ± 3°C for the finished product is considered to be justified.

Available data at 25±2°C/60±5% RH also supports instances of short-term excursions outside the label storage conditions. The company proposes an in use claim for storage outside the refrigerator for a single period up to 30 days, which is considered acceptable.

Adventitious agents

Pegvaliase is manufactured using an enzyme expressed in *E. coli* and no reagents or raw materials used in the production are derived from human or animal sources.

GMO

Not applicable.

2.2.4. 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 active substance and its manufacturing process.

However, no design spaces were claimed for the manufacturing process of the active substance.

At the time of the CHMP opinion, there were a number of minor unresolved quality issues having no impact on the Benefit/Risk ratio of the product.

2.2.5. 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. Data have been presented to give reassurance on viral/TSE safety.

2.3. Non-clinical aspects

2.3.1. Introduction

The non-clinical program was designed to support the chronic use of pegvaliase for PKU patients 16 years of age and older. The studies completed included single and repeat-dose pharmacodynamic (PD), safety pharmacology, toxicokinetic (TK) and toxicity evaluations in BTBRPahenu2 (ENU2) mice, and normal rats and monkeys. Developmental and reproductive toxicity studies were conducted in rats and rabbits. The pharmacological activity of pegvaliase was demonstrated in a rodent model of PKU, the ENU2 mouse. ENU2 mice exhibit HPA similar to PKU patients and were utilized to help determine initial dose levels of pegvaliase for clinical trials. All non-clinical studies used the subcutaneous (SC) route of administration.

2.3.2. Pharmacology

Primary pharmacodynamic studies

The ability of pegvaliase to cleave phenylalanine to trans-cinnamic acid (TCA) and ammonia was determined by measuring the increase in absorbance at 290nM as an indicator of the presence of TCA demonstrating that pegvaliase is able to convert phenylalanine to the specified constituent parts (Study QC-1104-A, see also Quality Section of this report).

The primary PD of pegvaliase was evaluated in the ENU2 mouse model. This mouse exhibits characteristics similar to those of PKU patients, including hyperphenylalaninemia (baseline plasma Phe concentrations of 1000 to 2000 μM/L) and hypopigmentation. Hypopigmentation is a phenotypic change caused by concentration-dependent Phe inhibition of melanin biosynthesis. Treatment of the hyperphenylalaninemia results in a progressive coat colour change in the ENU2 mouse.

In Study 0164-06-019, ENU2 Mice were administered either 0.2 or 1 IU avPAL, pegylated avPAL or 1 IU pegylated rtPAL (a pegylated PAL derived from Rhodosporidium toruiedise). Pegvaliase dose dependently decreased plasma phenylalanine and was more effective than either avPAL or rtPAL at any dose.

Similarly in Study 0165-06-057, weekly dosing up to 57 days using 0.25, 1 or 4 IU pegylatedAvPAL (wild-type and a mutant variant) in ENU2 mice resulted in a dose dependent decrease of serum phenylalanine. Plasma Phe levels decreased to less than 500 μM Phe in male ENU2 mice administered two weekly injections of the PEGylated AvPAL mutant. A transient attenuated response was seen during weeks 3 through 7 due to anti-drug-antibodies. Pharmacological activity returned after the eighth administration as evidenced by stable reduced levels of plasma Phe, below 500 μM over the entire week.

Weekly SC administration of pegvaliase at 80 mg/kg (approximately 4 IU/mouse) for 16 weeks reduced plasma Phe concentration from approximately 2000 μM/L to < 200 μM/L (Study 165-07-001). A transient attenuated Phe-lowering response was seen between Weeks 3 and 7, likely attributed to the antibody response to pegvaliase. However, from Week 7 onward, plasma Phe concentrations again decreased post-dose for the duration of the study. Repeated administrations of pegvaliase did not elicit signs of anaphylaxis or local injection-site reactions. No toxicities related to the lowering of plasma Phe were evident in the treated mice. In addition to lowering plasma Phe, the health of treated mice improved as evidenced by increased weight gain, increased lifespan, and fur coat color changes from light to dark brown.

Female ENU2 mice also have significant reproductive impairment as they are fertile but exhibit profound maternal PKU effects including impaired fetal outcome resulting in essentially 100% litter loss. Pups are born, but die within 24 hours of birth. A partial correction of maternal PKU syndrome is possible by feeding a Phe-restricted diet to pregnant female PKU mice; pups survive but are gradually lost until only 1 or 2 are alive at weaning.

Female ENU2 mice were treated with 5 or 10 mg/kg pegvaliase 3 times per week for up to 27 weeks and then daily with 6 or 7 mg/kg (Studies 165-09-068 and 165-10-008) which resulted in decreased plasma Phe levels from ~2000 µM/L to ~300-600 µM/L. After animals were switched to daily dosing, litter survival was 43% (6 out of 14 litters) with 4.3 pups per litter. Females gave birth to live pups, but only 2 out of 8 pregnancies successfully yielded live pups at weaning. These litters were also small compared to wild-type mice. Similar results were achieved in a subsequent study (0165-08-027) where Phe levels below 0.2 mM were reached.

The effects of pegvaliase on neuropathological parameters were also investigated in ENU2 mice. Mice received pegvaliase 3 times per week up to 12 weeks and were compared to age matched wild-type controls (Study 165-14-044). Phenylalanine decreased after administration of pegvaliase and showed a typical transient increase due to pegvaliase antibodies until tolerance was reached. Administration of pegvaliase also resulted in an upregulation of tyrosine hydroxylase and increased TH positive neurons in the dorsomedial hypothalamic nucleus and arcuate nucleus compared to ENU2 controls. While statistically significant, these changes were small compared to wild-type controls. No behavioural studies were conducted to assess whether the observed changes had functional effects.

Secondary pharmacodynamic studies

No secondary pharmacodynamics studies were submitted.

Safety pharmacology programme

The CNS effects of a single SC dose of 0, 10, 50, and 125 mg/kg pegvaliase administered to male and female rats were evaluated before dosing and at approximately 6, 24, 48, and 72 hours post-dose (Study 165-07-004). Each animal underwent a modified Irwin neurological assessment including a battery of behavioural tests and clinical observations. A reduction in body weight gain was seen in the high dose (125 mg/kg) group compared to control animals by Day 6 after dose administration. No changes in CNS parameters were noted in the pegvaliase-treated rats for any portion of the modified Irwin assessment at SC doses up to 125 mg/kg.

The respiratory effects of a single SC injection of 0, 10, 50, and 125 mg/kg pegvaliase were assessed in male rats (Study 165-07-005). Respiratory parameters included tidal volume, respiration rate, and minute volume. A single SC dose of up to 125 mg/kg of pegvaliase administered to male rats caused no alterations in respiratory function parameters evaluated in this study.

The CV effects of a single SC injection of 0, 1, 3, or 10 mg/kg pegvaliase were evaluated in conscious telemetry-instrumented cynomolgus monkeys (Study 165-07-006). Cardiovascular effects assessed were electrocardiogram (ECG) and hemodynamic (systolic, diastolic, and mean arterial pressures), inotropic state, heart rate, and abdominal temperature. No direct effects on cardiac rhythm, QT interval, or corrected QT interval (QTc) were observed at doses up to 10 mg/kg pegvaliase. No physiologically important changes were observed in hemodynamic data, inotropic state, heart rate or abdominal temperature.

Pharmacodynamic drug interactions

No pharmacodynamic drug interaction studies with pegvaliase were submitted.

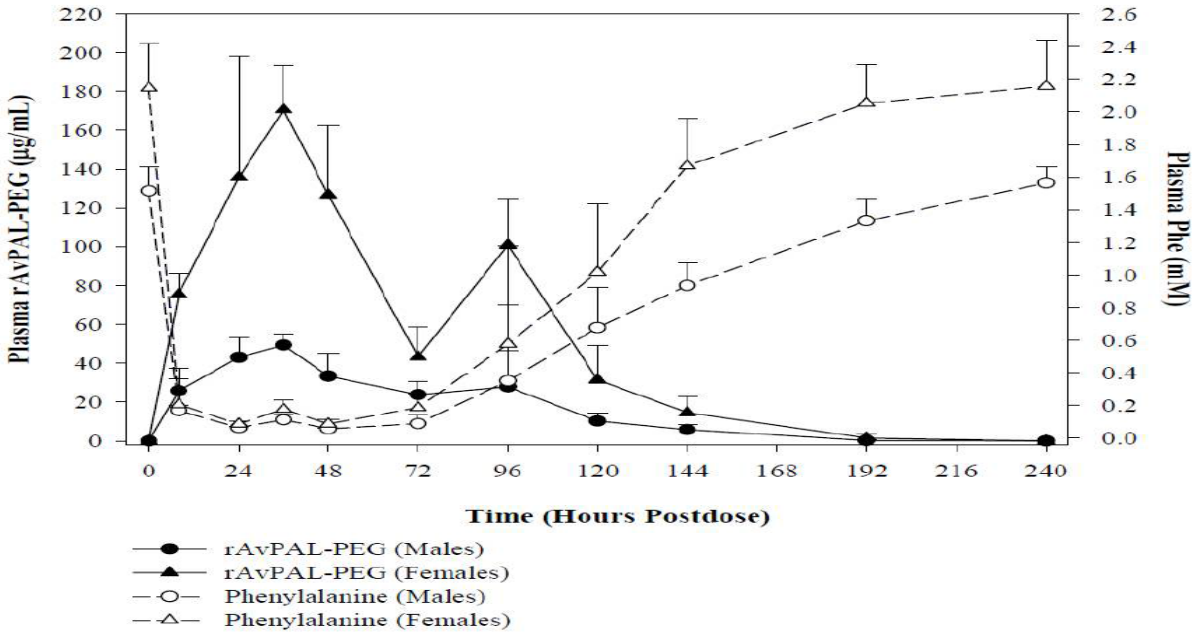
2.3.3. Pharmacokinetics

Absorption

Study 0165-07-028 was a single 20 mg/kg SC dose PK/PD study of pegvaliase in homozygote (-/-) male ENU2 mice. A Cmax of 209 µg/mL in plasma was observed at 24 hours post-dose, after which plasma concentrations of pegvaliase steadily declined with a terminal t1/2 of 26.9 hours (Vz/F and CL/F were 75.5 mL/kg and 1.95 mL/hr/kg, respectively). Plotting plasma pegvaliase against plasma Phe showed a PK-PD relationship of decreasing plasma Phe with increasing pegvaliase concentration. Plasma Phe levels reached the lowest levels at plasma pegvaliase concentrations ≥50 µg/mL.

Two studies (0165-09-069 and 0165-09-070) evaluated PK and PD of a single 20 mg/kg SC pegvaliase dose in homozygote (-/-) ENU2 male and female mice respectively. Thirty-two male and 28 female animals were administered pegvaliase and were divided into 10 groups of 2-4 mice/group based on schedule for terminal post-dose blood sampling procedures. Mean (SD) pegvaliase and Phe concentrations versus time for male and female rats are presented in **Figure 3**.

Figure 3. Mean (+SD) Pegvaliase and Phe Plasma Levels Following a Single 20 mg/kg SC Injection of Pegvaliase in Homozygote ENU2 Mice (Studies 0165-09-069 and 0165-09-070)



For both genders, all antibody titers remained below the assay cut point (<50 TU) except one male and three females having low titers of 50 across the time points investigated (8, 192 and 240 hours post-dose). Due to the low incidence of immunogenicity, impact on exposure could not be assessed.

Study 0165-07-003, was a single-dose PK of pegvaliase in male and female rats were determined with ascending single doses at 1, 5, and 25 mg/kg IV and 10, 25, and 250 mg/kg SC. This study included a 2-week recovery period.

Log-linear plasma plots from rats with IV injection showed terminal mono-exponential elimination and increased exposure with dose (data not shown). The SC plots showed similar characteristics as well as first-order absorption. PK parameters in this study are summarised in **Table 2.**

Table 2. PK Parameters in Rats Receiving Single Doses in Study 0165-07-003

Route of Administration	Dose mg/kg	AUC_{0-t} ng-hr/mL	C_{max} ng/mL	T_{max} hr	$t_{1/2}$ hr	CL[a] mL/hr/kg	V_{ss} mL/kg	V_z[a] mL/kg	F %
IV	1	606017	12600	4.5	28.3	1.55	72.1	64.1	NA
	5	3610522	87667	2.0	30.9	1.38	63.0	61.4	NA
	25	10856187	202238	9.0	41.7	2.34	145	139	NA
SC	10	1110433	16674	18.0	47.3	7.72	NA	532	16.9
	25	2292726	29260	42.0	33.4	10.5	NA	503	12.9
	250	34751389	206380	96.0	44.2	7.08	NA	449	7.79

NA, not applicable.

[a]For SC administration, clearance and volume of distribution are qualified by bioavailability, CL/F and Vz/F.

AUC0-t and Cmax dose plots forced through zero were roughly linearly proportional for IV and SC dosing and slighlty less than proportional at the highest dose (data not shown). An overlay of male and female plasma profiles qualitatively indicated no PK difference with gender.

Study 0165-07-007, was a single-dose PK study of pegvaliase in the monkey evaluating 3 doses, 4, 12, and 60 mg/kg, administered by SC injection. The log-linear plots showed increased exposure with dose and mono-exponential terminal elimination (data not shown). PK results from this study are summarised in **Table 3**.

Table 3. PK Parameters from Monkeys (SC, n=1) Receiving Single Doses in Study 65-07-007

Dose, mg/kg	AUC_{0-t}, ng-hr/mL (SD)	AUC_{0-24hr}, ng-hr/mL (SD)	C_{max}, ng/mL (SD)	T_{max}, hr (SD)	$t_{1/2}$, hr	CL/F, mL/min/kg	Vz/F, mL/kg
4	4625836 (1810469)	252874 (100086)	29337 (11017)	52.0 (20.7)	64.5[a]	0.820[a]	76.3[a]
12	20882173 (7690027)	518008 (149703)	147137 (65202)	106 (40)	71.3[a]	0.462[a]	45.6[a]
60	9319360 (6815626)	672490 (300228)	218387 (39058)	66.0 (36.2)	b	b	b

SD, standard deviation.

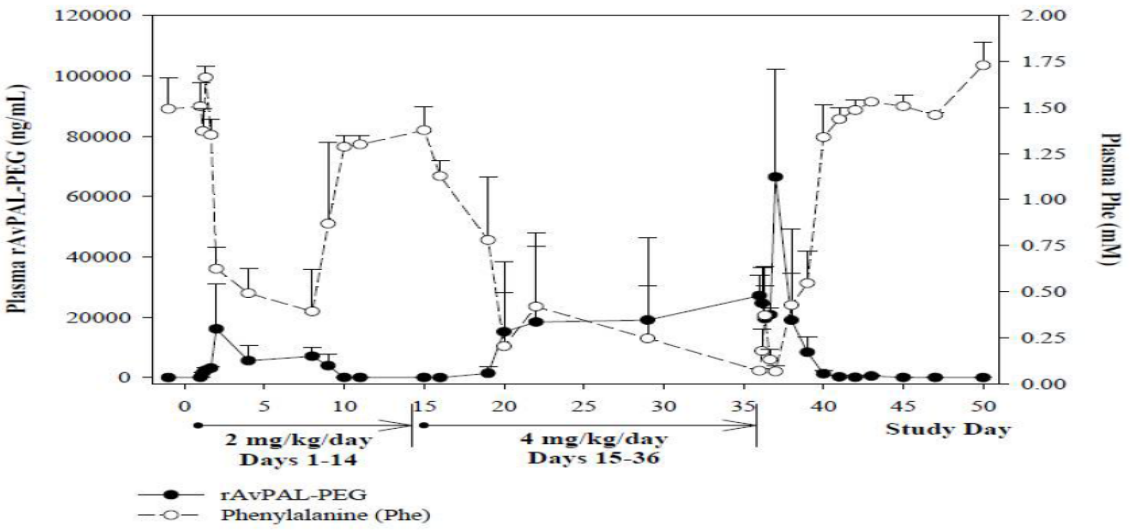
[a] n=1.

[b] Animals terminated early in the study.

An overlay of male and female plasma profiles qualitatively indicate no PK difference with gender. Regression of mean AUC0-t and Cmax values with the 4 and 12 mg/kg SC doses forced through zero indicated approximate linear proportionality; but less than proportional at the 60 mg/kg dose.

In study 0165-08-006 pegvaliase was administered SC once-daily, at 2 mg/kg/day from Days 1-14 and at 4 mg/kg/day from Days 15-36 with a 14 day recovery phase to investigate PK/PD effects in homozygote (-/-) male BTBRPahenu2 (ENU2) mice. Pegvaliase and Phe plasma concentrations over the course of Study 0165-08-006 are presented in **Figure 4**.

Figure 4. Overall Mean (±SD) Summary of Pegvaliase and Phe Plasma Levels Following Once-Daily SC Injections of Pegvaliase to Homozygote Male ENU2 Mice for 36 Days (study 0165-08-006)



Note: Pre-study sampling occurred on Day -14; for simplicity, predose data was presented as Day -1.

In Study 0165-07-009, Crl:CD(SD) rats, received SC injections of pegvaliase twice a week for 4 weeks, followed by a 2-week recovery period.

After the first dose, plasma exposure of pegvaliase in the rat increased with ascending doses. In Week 2 (Dose 4) plasma levels dropped below Week 1 for all 3 doses; in Week 4 (Dose 7) levels increased compared to Week 2 and for the high dose, levels returned to about the initial levels of Week 1. The change of pegvaliase plasma levels in Week 4 appears more pronounced with female rats than male (data not shown). PK parameters from this study are summarised in **Table 4**.

Table 4. PK Parameters in Crl:CD(SD) Rats over 28 Days (Study 0165-07-009)

Dose, (mg/kg)	Dose # (Day)	AUC_{0-t}, ng-hr/mL	C_{max}, ng/mL	T_{max}, hr
1	1 (1)	76388	1935	30.0
	4 (11)	2465	230	NA
	7 (22)	NA	NA	NA
8	1 (1)	1104773	26933	42.0
	4 (11)	4591	288	16.5
	7 (22)	158404	5143	22.5
25	1 (1)	2992269	72623	60.0
	4 (11)	878249	21064	24.0
	7 (22)	2755085	56430	36.0

PK parameters from male and female rats (combined) receiving pegvaliase SC injections twice weekly over 28 days.

NA, not applicable, concentration levels <LLOQ.

Study 0165-08-019 was a 26-week SC pegvaliase toxicity and TK study with a 12-week recovery period and a 17-week interim euthanasia with a 4-week recovery period in Crl:CD(SD) rats. **Table 5** presents mean PK parameter for 1, 8, and 25 mg/kg dosing at Weeks 1, 8, 16, and 27.

Table 5. Mean PK parameter in Crl:CD(SD) rats (n=9/sex/dose), Study 0165-08-019

		Pre-dose/ Dosing Phase[a]			Recovery Phase[b]		
Dose (mg/kg)	**Study Week**	**C_{max} (ng/mL)**	**T_{max} (hr)**	**$AUC_{0\text{-}72hr}$ (ng•hr /mL)**	**C_{max} (ng/mL)**	**T_{max} (hr)**	**$AUC_{0\text{-}72hr}$ (ng•hr /mL)**
1	**1**	1325	36	68029	NA	NA	NA
	8	276	0	4094	NA	NA	NA
	16	3085	36	65516	NA	NA	NA
	27	1994	48	67470	2008	24	147570
8	**1**	38129	48	1443872	NA	NA	NA
	8	44342	16	1617226	NA	NA	NA
	16	22446	60	1098690	NA	NA	NA
	27	18500	8	1036762	17099	72	2771088
25	**1**	128675	60	5552860	NA	NA	NA
	8	205500	4	9859624	NA	NA	NA
	16	106983	12	4781972	NA	NA	NA
	27	152944	36	6283204	139167	132	18637860
Additional Information:		PK Parameters reported are gender combined.					

[a] collection times for Weeks 1,8,16 and 27 were pre-dose (0), 8, 24, 48 and 72 hr.

[b] collection times for Week 27 were pre-dose (0), 24, 48, 72, 96, 120, 144, 168, 192, 216, 240, and 264 hr.

NA= Not applicable

All samples from all animals were negative for neutralizing antibodies to rAvPAL in rat serum through Day 272 of the dosing phase except for one male and one female given 1 mg/kg/dose. In both animals, neutralizing antibodies were only found on Day 120 of the dosing phase.

In Study 0165-07-008, Cynomolgus monkeys were dosed between 0.01 and 1 mg/kg over 4 weeks followed by a 4-week recovery period. As observed with single SC dosing, exposure pegvaliase increased with increasing doses in week 1 (Dose 1). For the second week, plasma levels increased giving an accumulation index of about 3.7 (1.1 to 14.4); time to steady state could not be determined from available data. These trends appeared to be similar for male and female monkeys. PK parameters from this study are summarised in **Table 6**.

Table 6. PK Parameters in Cynomolgus Monkeys, Study 0165-07-008

Dose, mg/kg	**Dose #**	**$AUC_{0\text{-}t}$, ng-hr/mL (SD)**	**C_{max}, ng/mL (SD)**	**T_{max}, hr (SD)**	**Accumulation Index, Dose 3/Dose 1 (SD)**
0.01	1	2318[a]	128[b]	42.0[b]	NA
	3	7890[a]	280[a]	36.0[a]	NA
0.1	1	30066 (14465)	689 (384)	33.0 (20.6)	NA
	3	70062 (40973)	1079 (787)	26.5 (19.7)	4.49 (5.59)
1.0	1	425366 (178337)	9873 (5372)	41.7 (13.7)	–
	3	1238924 (397836)	27781 (12193)	39.3 (29.2)	3.32 (1.67)

PK parameters from male and female monkeys (combined) receiving pegvaliase SC injections twice weekly over 28 days.

NA, not applicable, concentration levels <LLOQ; SD, standard deviation.

[a] n = 1

[b] n = 2

Study Number 0165-07-030 was a repeat-dose toxicity and TK study of pegvaliase in Cynomolgus monkeys. Plasma pegvaliase samples were collected through Week 39, with intensive PK collections taken from pre-dose to 72 hours post-dose following the first dose on Day 1 (Week 1) and last dose on Day 267 (Week 39). Summary statistics of PK parameters of pegvaliase after the first and last weeks of dosing are presented in **Table 7**.

Table 7. Summary of PK Parameters for Study No. 0165-07-030

		PK Parameters						
		First Week				Last Week (Week 39)		
Dose Group	Dose Leve (mg/kg)		C_{max} (ng/mL)	T_{max} (hr)	$AUC_{0\text{-}72\ hr}$ (ng•hr/mL)	C_{max} (ng /mL)	T_{max} (hr)	$AUC_{0\text{-}72\ hr}$ (ng•hr/mL)
2	0.01	Mean	51	43.2	2776	0.0	NA	0.0
		SD	29	20.1	1818	0.0	NA	0.0
		N	6	5	6	6	0	6
3	0.1	Mean	908	57.6	45053	81	12.8	996
		SD	761	21.5	39358	132	7.5	1895
		N	6	5	6	6	4	6
4	1.0	Mean	23427	72	928020	637	18.0	28431
		SD	11523	0	237747	476	15.9	24209
		N	6	6	6	6	6	6
5	3.0	Mean	47654	49.7	2334176	21831	18.6	1205170
		SD	23286	14.8	916955	50391	12.0	2854258
		N	14	14	14	14	14	14
6	7.0/5.0/3.0[a]	Mean	144598	53.1	6122640	3644	23.1	144935
		SD	41582	13.9	1814405	4367	13.0	177175
		N	14	14	14	13	13	13
Additional Information:		PK parameters reported are gender combined.						

[a] Dose for this group was reduced due to toxicity from 7.0 to 5.0 to 3.0 mg/kg; NA=Not Applicable

All control animals and animals given 0.01 mg/kg/dose remained negative for neutralizing antibodies. All other animals, with the exception of one female given 0.1 mg/kg/dose and one female given 7.0 mg/kg/dose (unscheduled early sacrifice), became positive for neutralizing antibodies, however, a correlation between the anti-pegvaliase IgG antibodies and neutralizing antibodies to pegvaliase was uncertain due to the relatively small number of subjects with positive neutralizing antibodies.

Metabolism and Excretion

No metabolism or excretion studies with pegvaliase were submitted.

Pharmacokinetic drug interactions

No pharmacokinetic drug-drug interactions with pegvaliase were submitted.

Immunogenicity

In all repeat-dose toxicity studies and some of the developmental toxicity studies, the presence of anti-drug antibodies (ADA) was monitored.

In the 4-week study in rats and the 4- and 39-week study in monkeys, anti-rAvPAL-PEG IgG was monitored. Overall, an IgG response was noted in all dose groups, starting at 2-3 weeks of the study. In rats, approximately 50% of the animals showed anti-rAvPAL-PEG IgG antibodies at the end of the dosing period; in monkeys, all animals became positive in the 39-week study, as well as all high dose and some low/mid dose animals in the 4-week study.

In the 26-week study in rats, the fertility and developmental study in rats and the dose range finding developmental study in rabbits, anti-rAvPAL IgG was monitored. These antibodies were first observed 2 weeks after dosing. In the 26-week study in rats, 50-78% of the rats were positive for anti-rAvPAL IgG at the end of the dosing period (more animals at lowest dose). However, in the fertility study in rats, only 36% of the low dose females and 16-52% of the males became positive. Also in the rabbit developmental (range finding) study, only 20% of the animals (low and mid dose) showed an antibody response. It is noted that plasma drug levels for animals at the end of the study were (at least in several animals) greater than assay drug tolerance (2 µg/mL).

rAvPAL-IgM was monitored in the 4-week studies in rats and the 4- and 39-week studies in monkeys. rAvPAL-IgM was observed in none of the monkeys and two of the rats in the 4-week study and in several of the monkeys at the end of the 39-week study (including some of the control females).

Neutralizing ADA's, diminishing the activity of pegvaliase were observed in two rats of the 26-week repeated dose study (and one male in the 17-week interim analysis) and in 40/48 monkeys of the 39 week repeated dose study (none in the lowest dose group).

2.3.4. Toxicology

Single and repeat dose toxicity studies of SC administered pegvaliase were conducted in rats and monkeys. Single dose studies are summarised in **Table 8**, and repeat dose studies in **Table 9**.

Single dose toxicity

Table 8. Single dose toxicity studies with pegvaliase

Study ID	Species/ Sex/Number/ Group	Dose/Route	Approx. lethal dose / observed max non-lethal dose	Major findings
0165-07-003	Rat	IV: 0-1-5-25 SC:0-10-25-250	ND/250	NOAEL at 25 (IV) and 250 (SC) **Mortality** 5IV:1M (technical) 10SC: 1M (technical) **Clinical observation** =250:↓BW, ↓BW gain, red or swollen skin, rough coat
0165-07-007	Monkey	*0-4-12-60 SC*	>12<60/12	**Mortality** =60: All animals; depression, dehydration, anorexia, hypothermia/cold to touch, hunched posture, lump at injection site, hypoactivity, vomitus containing food, squinting eyes, rough hair coat **Clinical observations** ≥4: Diarrhoea ≥60:↓BW **Clinical chemistry** ≥4: ↓Phe, ≥12: ↓Retic, ↓MCV =60: ↓Lymph, ↓Eos, ↓Tprot (↓alb, ↓glob), ↓ALP, ↑BUN **Gross pathology** =60: stomach pyloric ulceration, red or red and raised areas at ileocecal junction, cecum and colon. **Histopathology** =60: mucosal atrophy, congestion and haemorrhage, degeneration and necrosis of glandular epithelium, erosion and/or ulceration, and subacute inflammation

Repeat-dose toxicity

Table 9. Repeat-dose toxicity studies with pegvaliase

Study ID	Species /Sex/ Number /Group	Dose/Route	Duration	NOEL/ NOAEL (mg/kg /day)	Major findings
0165-07-009	Rat/ 10-15/s/ g	0-1-8-25 BIW SC	4 weeks	1/8	**Histopathology** ≥8F: Vacuolation of spleen ≥8: minimal to marked fibrosis at injection site with minimal to slight lymphocyte/macrophage infiltration =25M: Vacuolation of spleen **Recovery** Vacuolation of spleen
0165-08-019	Rat/ 25-35/s/ g	0-1-8-25 BIW SC	26 weeks	1/8	**Mortality** =1: 1M malocclusion, 2M moribund **Clinical observation** ≥8: thickened injection site =25F: thinning coat =25: ↓BW, ↓BW gain **Serum chemistry** =25M: ↓urine pH =25:↓Trig, ↓ALT (≥8 in F) **Histopathology** ≥8: focal to multifocal vacuolation/hypertrophy of kidney tubules, histiocytic vacuolation in liver, spleen, mesenteric lymph node, mandibular lymth node (males only @25) =25: histiocytic valuolation adrenal cortex =25M: histiocytic vacuolation testes **Recovery** Vacuolation/hypertrophy of kidney tubule, Vacuolation of histiocytic cells

0165-07-008	Cynomolgus monkey/ 3-5/s/g	0-0.01-0.1-1 BIW SC	4 weeks	0/0.01	**Serum chemistry** ≥0.1: ↓Phe **Histopathology** ≥0.01: mild to moderate perivascular infiltrate in subcutus at injection site ≥0.1: Kuppfer cell hypertrophy, Minimal to slight degeneration of blood vessels in various tissue (lung, stomach, gallbladder, kidney, colon, pancreas, spleen, prostate) ≥0.01M: pigment in kidney tubules
0165-07-030	Cynomolgus monkey/ 3-8/s/g	0-0.01-0.1-1-3-7/5/3 BIW SC	39 weeks	0.1/1	**Mortality** =7: 1F (anorexia, poor body condition) **Dose adjustment due to adversity in F** =7: suspended on d11, to 5 on d18, suspended on d22 to 3 on d 46. Days without dosing: 31 Adversity included: low or no food consumption, body weight loss, and/or hypoactivity **Clinical observation** ≥3F: emesis ≥3:liquid faeces, **Serum chemistry** ≥0.1: ↓Phe **Histopathology** ≥0.1: Kidney glomerulopathy ≥3: arterial inflammation (incl heart), perivascular cellular infiltrates at injection site, renal glomerulopathy, thymus atrophy, monocellular infiltrate lacrimal gland, heart infiltrate (myocardium), lymphoid nodules in sternum bone marrow **Reversilibility** ≥3: emesis (F), liquid faeces, Thymic atrophy, lymphoid nodules
BMN165-13-047 (**non-GLP**)	Rat or Cynomolgus Monkey	Rat: 0-1-8-25 BIW SC Monkey: 0-0.01-0.1-1 BIW SC	Rat 26 weeks Monkey 39 weeks		Assessment of potential PEG staining and vaciolation in brain tissue and choroid plexus of the pivotal rat and monkey studies No binding to any assessed tissue was observed for any group

				IHC validation of antiPEG and anti rAvPAL PEG - Used samples of 019 =25: Staining in renal tubular epithelium and in infiltrating or fixed phagocytes in adrenal gland, liver, lymph node (mandibular and mesenteric), and spleen. No binding to brain tissue
BMN165-13-069	Rat	0-1-8-25 BIW SC	4-17-26 weeks	ADA binding IHC study based on 009 and 019 ≥8: ADA staining in blood vessels and kidney epithelia. Steady state accumulation of rAvPAL-PEG in kidney

Genotoxicity

No genotoxicity studies with pegvaliase were submitted.

Carcinogenicity

No carcinogenicity studies with pegvaliase were submitted.

Reproduction Toxicity

A summary of the main finding from the reproductive and developmental toxicology studies conducted with pegvaliase is presented in **Table 10**.

Table 10. Overview of reproductive and developmental toxicology studies with pegvaliase

Study type/ Study ID / GLP	**Species; Number Female/ group**	**Route & dose**	**Dosing period**	**Major findings**	**NOAEL (mg/kg &C_{trough})**
Fertility/EFD BMN 165-12-037	Rat; 25/s/g	0-2-8-20 mg/kg/d SC	6 weeks and up to GD17	=20: ↓FC, ↓BWgain, ↓BW Serum chemistry ≥8: ↓Phe Pathology =20F:↓absOvary weight, ↓relLeft ovary weight **Fertility** ≥8F: ↓corpora lutea, =20F:↓litter size and live litters, ↓foetal weight **F1** =20: ↑large structures (nasal-frontal), incompletely ossified squamosal bones, frontal bones, arches in the lumbar	F0 8 (F: 57800 ng/mL on GD21; M: 20400 ng/mL DS79) F1 2: 9280ng/mL

				vertebrae, ribs and pubes, ↓ossified sternal centers and metacarpals per fetus per litter ↓ossified metatarsals per fetus per litter	
EFD BMN165-12-036	Rabbit; 10F/g	0-2-5 mg/kg/d SC	GD7-20	**F0** ≥2F:↓FC =5F: Thin body, sparse hair coat, dehydration, ↓BWgain, ↓BW(GD11-16) increased abortion **F1** ≥2:↑Malformations ↑Variations =5: ↑embryofoetal death (GD7-12), **Foetal anomalies**: ≥2: skeletal variations, Cleft palate, eye lids open, =5: Oedema, flexed or short or rotated fore and/or hind limbs, short snout, cleft palate, micrognathia jaw, open eyelids, enlarged eyes, low set ears, rotated ears, domed head, exencephaly, meningocele, malformed digits, absent gallbladder, small lungs, absent or small kidneys, protruding tongue	ND
PPND BMN165-14-013	Rat; 25F/g	0-2-8-20 mg/kg/d SC	4wksPC-L21 dd SC	F0 ≥20:↓FC, ↓BW F1 ≥20:↓survival to d4, ↓survival to weaning, ↑litterloss, ↓pup weight, ↑prepuital separation F2 =8: 1 foetus with misshaped snout, mall oral opening and eye, bulge depressed ≥20: ↑foetal BW	8 (98633 ng/mL)

Toxicokinetic data

Exposure levels of pegvaliase from the nonclinical studies are listed in Table xx and compared to exposures in humans from current clinical trials.

Table 11. Exposure Comparison between Animal and Human Phase 3 Data

Species	Study	Dose	Week	[a]AUC0-24hr (hr*ng/mL)	[a]AUC0-72hr (hr*ng/mL)	Cmax (ng/mL)	Ctrough (ng/mL)
Rat	0165-08-019	[b]1 mg/kg (twice weekly)	Week 1	NA	68029	1325	NA
			Week 8	NA	4094	276	NA
			Week 16	NA	65516	3085	NA
			Week 27	NA	67470	1994	NA
Monkey	0165-07-030	[b]1 mg/kg (twice weekly)	Week 1	NA	928020 (237747)	23427 (11523)	NA
			Week 39	NA	28431 (24209)	637 (476)	NA
Human	165-302	20 mg/day	Part 3 Week 1	143039.7 (174109.69)	NA	7098.4 (7971.62)	6464.8 (6841.81)
		40 mg/day	Part 3 Week 1	206572.3 (212342.03)	NA	10653.4 (13438.66)	9727.3 (10438.97)
		20 mg/day	Part 3 Week 5/6	262181.5 (280378.19)	NA	14042.6 (16254.69)	11177.3 (8974.65)
		40 mg/day	Part 3 Week 5/6	246782.5 (338587.54)	NA	16687.3 (19457.12)	10446.0 (12723.89)

[a] Mean(SD)

[b] Previously determined no observed adverse effect level

AUC, area under the curve; NA, Not available

2.3.5. Ecotoxicity / environmental risk assessment

Pegvaliase is a PEGylated protein product, prepared using recombinant DNA technology comprised of naturally occurring amino acids that is administered for the treatment of adult patients only with PKU. The applicant considered that pegvaliase does not present any risk for the environment following its prescribed use in patients.

2.3.6. Discussion on non-clinical aspects

The ability of pegvaliase to cleave phenylalanine to trans-cinnamic acid (TCA) and ammonia was determined by measuring the increase in absorbance at 290nM as an indicator of the presence of TCA demonstrating that pegvaliase is able to convert phenylalanine to the specified constituent parts.

A number of *in vivo* studies were conducted with pegvaliase in BTBRPahenu2 (ENU2), a homozygous mutant at the phenylalanine hydroxylase gene (PAH) locus. This mouse model exhibits similar clinical characteristics of PKU patients including hyperphenylalanemia, hypopigmentation and cognitive deficits.

Overall, *the in vivo* studies demonstrate that in ENU2 mice, pegvaliase is able to dose dependently reduce phenylalanine, improves neurological effects, increases the weight and restores coat colour. In female mice, improvements on neonatal survival were achieved although the relevance for this observation in humans is limited. Despite the profoundly reduced phenylalanine levels, the changes in phenotype of ENU2 mice were modest.

Administration of a single dose pegvaliase up to 125 mg/kg in rats did not result in any neurological changes. A reduction in body weight was observed in the high dose group by day 6 without noticeable changes in CNS parameters. There were no effects on tidal volume, respiration rate, and minute volume.

There were no effects on cardiac rhythm, QT interval, or corrected QT interval (QTc), hemodynamic data, inotropic state, heart rate or abdominal temperature after a single dose of pegvaliase up to 10 mg/kg in monkeys.

Based on the pharmacology studies submitted by the applicant, pegvaliase appears to have a low potential to adversely affect the CNS, respiratory system or cardiovascular system.

No secondary PD studies were submitted and this was considered acceptable, as pegvaliase is intended as an enzyme substitution therapy.

No pharmacodynamic drug interaction studies were submitted, as based on the structure of the product (recombinant bacterial enzyme) it is not expected that pegvaliase would interact with drug metabolizing enzyme systems.

Single dose pharmacokinetic with SC doses of 4, 12 and 60 mg/kg in the monkey and 10, 25 and 250 mg/kg in the rat were performed. In the rat study, I.V. single doses (1, 5 and 25 mg/kg) were also included to determine bioavailability. In addition, single dose studies were performed in ENU2 mice.

In general, the absorption of pegvaliase, following SC administration, was slow in rats and monkeys, with a Tmax of 18-96 and 52-105 hours, respectively. Pegvaliase showed a mono-exponential elimination. No gender differences were observed in these species. The volume of distribution (Vz/F) was 449-532 mL/kg in rat and 45.6-76.3 in monkey, compared to 317-370 mL/kg in humans (22.2-26.4L). Plasma clearance was 7.1-10.5 mL/hr/kg in rat, but much lower in monkeys (0.46-0.82 mL/hr/kg). Data in monkeys (T1/2, Vz and CL) are based on 1 animal in the low dose and 1 animal in the mid dose. Systemic exposure of pegvaliase increased in a roughly linear dose proportional manner at the lower dose levels, but appears to be less than proportional at the highest dose level in both rat and monkey. Terminal elimination (T1/2) following SC injection was slow (33-47 hours in rats and 64-71 hours in monkeys) and independent of increasing dose.

The mean SC bioavailability of pegvaliase in rats was 7.8-16.9%, when compared to the 1 mg/kg IV dose and decreased with increasing SC dose levels. It is noted that when the SC doses are compared to the 25 mg/kg I.V. dose, bioavailability ranged between 12-35%.

Studies in ENU2 mice (using Phase 2 Process A material) showed gender differences: AUC and Cmax were approximately 3.1- and 3.5-fold higher, respectively, in females compared to males, whereas the apparent volume of distribution (Vz/F) and systemic clearance (CL/F) for pegvaliase in males were approximately 2.2- and 3.1-fold higher than females. In addition, exposure (AUC, Cmax) using Phase 1 pegvaliase material in males was approximately 3-4 fold higher than the Phase 2 Process A material.

No metabolism studies with pegvaliase were conducted in animals. The metabolism of phenylalanine ammonia lyase is expected to occur via catabolic pathways and to be degraded into small peptides and amino acids.

Due to the nature and molecular size of pegvaliase, no renal or hepatic excretion is anticipated. Therefore, no specific studies to measure excretion of pegvaliase were conducted. The absence of metabolism and excretion studies is in accordance with ICH S6(R1) and was considered acceptable by the CHMP.

PK drug-drug interactions at are highly unlikely for pegvaliase as biotechnology-derived substances are not metabolized via CYP P450 enzymes. The absence of such studies was therefore considered acceptable.

Single and repeat dose toxicity studies of SC administered pegvaliase were conducted in rats and monkeys and included a 2-3 week recovery period. Lethal doses in monkeys occured between 12 and 60 mg/kg, whereas no drug-related lethality was observed in rats given up to 250 mg/kg pegvaliase.

Multiple toxico-kinetic studies were performed in rats (4 and 26 weeks; dose levels 1-25 mg/kg) and Cynomolgus monkeys (4 and 39 weeks; dose levels 0.01-7/5/3 mg/kg) upon twice weekly SC administration as well as in Enu2 mice (36 days, daily SC administration of doses of 2/4 mg/kg).

In female rats vacuolation of the spleen was observed at the 8 or 25 mg/kg pegvaliase dose, but only in the high dose in males. This finding was still observed in the recovery group. In the 26 week study, histiocytic vacuolation of the spleen, liver, kidney tubules, mesenteric lymph node and mandibular lymph node was observed in in females from 8 mg/kg biw upwards and in males from 25 mg/kg. Vacuolation of

the adrenal cortex was observed in both males and females given 25 mg/kg pegvaliase. Vacuolation of the testes was observed in males given 25 mg/kg pegvaliase. In the recovery group, minimal vacuolation of histiocytic cells persisted in these organs, but at a lower incidence and severity, suggesting partial reversibility.

Vacuolation is likely an adaptive response to clearance of PEG, especially in the kidney, which is the target organ for the clearance of low molecular weight (<20kDa) PEG and was also associated with increased renal tubular hypertrophy. No vacuolation was observed in the brain or choroid plexus (CP) of rats and no vacuolation was observed in the monkey in any tissue. The applicant considered that tissue vacuolation reflects tissue distribution of PEG. However, no separate group of animals was treated with PEG alone in order to make meaningful comparisons of potential PEG-related effects. The vacuolation observed in these studies was not associated with any organ related toxicities as determined by clinical chemistry/urinalysis and histopathological analysis.

In the repeat dose studies plasma rAvPAL-PEG values increased after the first doses, with a transient drop after 2-4 weeks. This drop coincides with the peak in anti-PEG IgM/IgG and anti-PAL IgM antibody responses, circulating immune complex (CIC) levels and complement activation and may be explained by a combination of rapid clearance mediated by the early anti-PEG antibody response and relatively high levels of IgM (both forming immune complexes and activating complement), and not by anti-PAL IgG antibodies, which are less likely to activate complement.

This is supported by the non-clinical immunogenicity data. In most of the studies, only anti-PAL antibodies were measured. However, In the 4 week study in monkeys, however, anti-PEG IgG was measured. At day 28, anti-PEG IgG was detected in 5/6, 3/6 and 2/6 animals of the 0.01, 0.1 and 1 mg/kg dose, respectively. A dose-dependent decrease in pegvaliase levels was observed around day 22 in all dose groups.

In cynomolgus monkeys, the incidence and severity of arterial inflammation was dose dependant and observed in a wide range of organs and tissues at clinically relevant exposures in the 4-a dn39-week repeat-dose toxicology studies. The arterial inflammation observed in these studies involved small arteries and arterioles in a wide range of organs and tissues and in subcutaneous injections sites. Arteritis was attributed to the immune-mediated response with chronic administration of foreign proteins to the animals. The vascular inflammation observed in these studies was reversible upon cessation of treatment.

Dose-dependent reductions in body weight gain attributed to decreased plasma phenylalanine levels to below normal levels in normal animals (monkeys, rats and rabbits) was observed in single and repeat dose toxicology studies as well as developmental and reproductive toxicity studies with pegvaliase. Decreased plasma phenylalanine and reduced body weight gain was reversible after cessation of treatment.

Adverse reproductive and developmental effects of pegvaliase in rats and rabbits were dose dependent and included reduced implantation rate, smaller litter size, lower foetal weights, and increased foetal alterations. Additional findings in rabbits included increased abortions, foetal malformations and embryo/foetal lethality. These findings occurred in the presence of maternal toxicity (decreased body weights, decreased ovarian weights, and decreased food consumption) and were associated with markedly decreased maternal blood phenylalanine below normal levels in non-PKU animals. The contribution of maternal phenylalanine depletion to the incidence of embryo-foetal developmental effects was not evaluated.

In the peri/postnatal study, pegvaliase decreased pup weight, litter size, and survival of offspring during lactation, and delayed sexual maturation of offspring when administered daily in rats at 20 mg/kg subcutaneously. The effects in offspring were associated with maternal toxicity.

Foetal toxicity is included in the Risk Management Plan as an important potential risk and will be further characterised by a Peri-/postnatal development study in rats and a foetal development study in rabbits. Uncontrolled blood phenylalanine levels (hyperphenylalaninaemia) before and during pregnancy are associated with increased risk for miscarriage, major birth defects (including microcephaly and major cardiac malformations), intrauterine foetal growth retardation and future intellectual disability with low IQ.

In case of hypophenylalaninaemia during pregnancy, there is a risk of intrauterine foetal growth retardation. Additional risk to the unborn child due to hypophenylalaninaemia is not established.

Maternal blood phenylalanine levels must be strictly controlled between 120 and 360 µmol/l both before and during pregnancy. Palynziq is not recommended during pregnancy, unless the clinical condition of the woman requires treatment with pegvaliase and alternative strategies to control phenylalanine levels have been exhausted.

Available toxicological data in animals have shown excretion of pegvaliase in milk. It is unknown whether pegvaliase is excreted in human milk. In the pups of these animals, systemic exposure of pegvaliase was not detected. A risk to infants cannot be excluded. Due to lack of human data, pegvaliase should only be administered to breast-feeding women if the potential benefit is considered to outweigh the potential risk to the infant.

Pegvaliase is a recombinant protein and no genotoxicity is expected from these types of products. The CHMP considered that the absence of genotoxicity studies was acceptable.

No carcinogenicity studies with pegvaliase were submitted, as it is not expected to bind to DNA. The applicant also performed a literature search which did not reveal any evidence of carcinogenicity for PAL, rAvPAL or PEG. The absence of carcinogenicity studies was considered acceptable by CHMP.

The active substance is a natural substance, the use of which will not alter its concentration or distribution in the environment. Therefore, pegvaliase is not expected to pose a risk to the environment and the lack of a detailed environmental risk assessment was considered acceptable by CHMP.

2.3.7. Conclusion on the non-clinical aspects

The pharmacologic, pharmacokinetic, and toxicological characteristics of pegvalaise have been adequately characterised from a non-clinical perspective. A peri-/postnatal development study in rats and a foetal development study in rabbits is expected to provide information on maternal phenylalanine depletion in the incidence of embryo-foetal developmental effects.

2.4. Clinical aspects

2.4.1. Introduction

GCP

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.

- Tabular overview of clinical studies

Type of Study (identifier)	Objective(s) of the Study	Study Design and Type of Control	Number of Patients	Healthy Subjects or Diagnosis of Patients	Duration of Treatment
Phase 1: Safety, tolerability, and dose optimization (PAL-001) Study Complete; Final CSR	To assess the safety and tolerability of single, SC injections of rAvPAL-PEG in subjects with PKU. To evaluate the PK of single, SC injections of rAvPAL-PEG administered at escalating doses in subjects with PKU and to evaluate the effect of rAvPAL-PEG on blood phenylalanine concentrations in subjects with PKU.	Open-label, Dose-Escalation Study to evaluate a Single dose	planned: ~35 enrolled: 25 (5 per dose group) completed: 25 active: 0	16 to 50 years old patients with PKU	43 days (1 day of treatment + 42 days of follow-up)
Phase II: Safety, efficacy, and tolerability (PAL-002) Study Complete; Final CSR	To evaluate the effect of multiple doses of rAvPAL-PEG on blood phenylalanine concentrations in subjects with PKU for up to 16 weeks of treatment. To evaluate the safety and tolerability of SC injections of multiple dose levels of rAvPAL-PEG in subjects with PKU. To evaluate the antibody response to rAvPAL-PEG in subjects with PKU. To evaluate the PK profile of rAvPAL-PEG in subjects with PKU. To evaluate the safety, PK profile, antibody response, and blood phenylalanine concentrations of rAvPAL-PEG-1 compared with rAvPAL-PEG-2.	Open-label, Dose-Finding Study to evaluate multiple doses	planned: ~55 enrolled: 40 completed: 37 active: 0	16 to 55 years old with PKU	Up to 16 weeks
Phase II: Efficacy, safety, and tolerability (PAL-003) Study Ongoing CSR (data cut off February 2018)	To evaluate the effect of long-term administration of multiple SC injections of rAvPAL-PEG on blood phenylalanine concentrations in subjects with PKU. To evaluate the safety and tolerability of long-term administration of SC injections of rAvPAL-PEG in subjects with PKU. To evaluate the immune response to long-term 1administration of SC injections of rAvPAL-PEG in subjects with PKU.	Long-term extension, Open-label, Study to evaluate multiple doses of pegvaliase in subjects who completed PAL-002, PAL-004 or 165-205	Planned: ~100 enrolled: 68 completed: 0 active: 44	Patients with PKU	Up to 86 months or until study is terminated
Phase II: Efficacy, safety, and tolerability (PAL-004) Study Complete; Final CSR	To evaluate the effect of daily administration of pegvaliase on the reduction of blood phenylalanine concentrations in subjects with PKU. To evaluate the safety and tolerability of SC injections of daily administration of rAvPAL-PEG in subjects with PKU. To evaluate the antibody response to rAvPAL-PEG administered daily to subjects with PKU.	Open-label to evaluate multiple doses	enrolled: 16 completed: 15 active: 0	Patients with PKU 16 to 70 years	13 weeks
Phase II: Safety, efficacy, PK (165-205) Study Complete; Final CSR	To identify a safe and efficacious dosing regimen of pegvaliase by evaluating multiple schedules involving bolus, induction, titration, and maintenance dosing. To evaluate immune response of multiple SC doses of rAvPAL-PEG on reduction of blood phenylalanine concentrations in patients with PKU. To evaluate PK profile of multiple SC doses of rAvPAL-PEG on reduction of blood phenylalanine concentrations in patients with PKU.	Multi-center, Open-label, Dose-finding Study to evaluate multiple schedules involving bolus, induction, titration, and maintenance dosing	planned: ~24 completed: 24 active: 0	Patients with PKU 16 to 70 years of age	24 weeks

Phase III: Safety and efficacy (165-301) Study Complete; Final CSR; study was early terminated by applicant	To characterize the safety and tolerability during induction, titration, and maintenance dosing in pegvaliase-naïve subjects who self-administer pegvaliase at dose levels of 20 mg/day and 40 mg/day. To evaluate blood phenylalanine concentration during induction, titration, and maintenance dosing in pegvaliase-naïve subjects who self-administer pegvaliase at dose levels of 20 mg/day and 40 mg/day.	Multi-Center, Open-label, Randomized, Safety and Tolerability Study to evaluate 2 doses (20 mg/day and 40 mg/day) (subjects feed into Study 165-302 to complete the placebo-controlled randomized discontinuation study)	planned: ~300 enrolled: 261 transitioned to Study 165-302: 203	Patients with PKU naïve to pegvaliase 18[a] to 70 years of age	Up to 36 weeks
Phase III: Efficacy (165-302) Study ongoing Interim CSR (clinical data cut off 5 February 2018)	To evaluate blood phenylalanine concentration in previously exposed subjects who self-administer pegvaliase (40 or 20 mg/day) compared with previously exposed subjects who self-administer matching placebo. To evaluate inattention and mood symptoms in subjects who self-administer pegvaliase (20 or 40 mg/day) compared with previously exposed subjects who self-administer matching placebo.	Four-Part, randomized, Double-blind, Placebo-controlled, 4-Arm, Discontinuation Study to evaluate 2 doses in subjects who previously completed Study 165-301.	planned: ~250 enrolled: 215 active: 180	Patients with PKU 18[a] to 70 years of age previously treated with pegvaliase.	Up to 212 weeks
Phase III: Executive Function (165-303) Study Complete; Final CSR	To evaluate executive function in adult subjects with PKU who are concurrently participating in Study 165-302, as measured by selected CANTAB tasks. Exploratory-To evaluate patient self- perception in adult subjects with PKU who are participating in Study 165-302 for use as anchors in the interpretation of change over time in measures of inattention and mood.	Phase III sub-study (no control)	planned: 100 enrolled: 9 completed: 9 active: 0	patients with PKU 18[a] to 70 years of age	63 weeks

CANTAB, Cambridge Neuropsychological Test Automated Battery; CSR, Clinical Study Report; PFS, prefilled syringe product presentation; VS, vial and syringe product presentation; SC, subcutaneous.
[a] The study age criterion was modified with a protocol amendment to increase the lower age limit from 16 years old to 18 years old per feedback from the Food and Drug Administration (FDA Advice/information request; 14MAY2014).

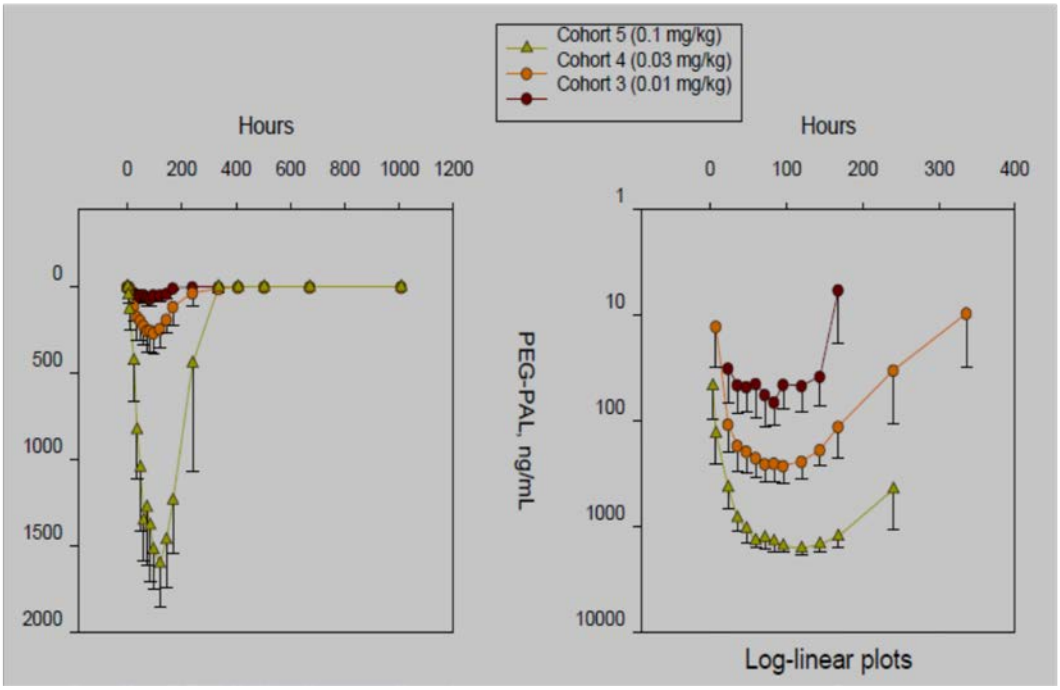
2.4.2. Pharmacokinetics

Absorption

- **Bioavailability**

Single dose PK of pegvaliase was studied in patients with phenylketonuria (PKU) in study PAL-001 (Phase I). Subjects (n=5 per cohort) were administered 0.001, 0.003, 0.01, 0.03, and 0.1 mg/kg doses, respectively. Subjects were to receive a single dose (i.e. no intra-subject repeated dosing or dose escalation) as a subcutaneous (SC) injection and then were to be followed for a total of 43 days (6 weeks) with visits to the clinical research unit. No exposure was observed at the 0.001 and 0.003 mg/kg dose. The mean Tmax values at the three doses 0.01, 0.03 and 0.1 mg/kg were 89, 106 and 101 hrs; the mean half-lives are at 59.5, 45.8 and 120 hr, respectively. The mean concentration-time curves for dose 0.01, 0.03 and 0.1 mg/kg are presented in **Figure 5**.

Figure 5. Blood Phe versus pegvaliase concentration over time for subjects (0.01, 0.03 and 0.1 mg/kg). (Study PAL-001)



- **Bioequivalence**

Study drug was manufactured using 4 different processes (A, B, C, and D) over the course of the program below (**Table 12**).

Table 12. Different manufactured Pegvaliase and devices used in clinical studies.

Clinical Phases	Study number	Study drugs	Devices
Phase I	PAL-001	Process A	Vial
Phase II	PAL-002	processes A and B	Vial
	PAL-004	process B	Vial
	165-205	processes C and D	Vial
	PAL-003	processes B, C and D	Vial and prefilled syringe
Phase III	165-301 and 165-302	Process D	Vial and prefilled syringe

Bioequivalence has been investigated between drug with Process A and B (study PAL-002) which showed comparability in PK of pegvaliase between them. As process B contained increased PEG, this result indicated that increased number of PEG does not improve the exposure of pegvaliase. Processes C and D contained the same number of PEG as Process A but with two different presentations of pegvaliase, i.e. vial/syringe (VS) and pre-filled syringe (PFS, the final commercial product).

- **Influence of food**

The influence of food on absorption of pegvaliase has not been studied.

Distribution

The apparent volume of distribution (Vz/F) of pegvaliase has been estimated in PKU patients in study 165-302 (Part 3 PK data) with the doses of 20 mg and 40 mg daily. Mean apparent volume of distribution (SD) of pegvaliase were calculated based on limited patients who were treated with PFS (excluding the subject who switched from placebo to treatment arm), and were found to be 26.4 L (±64.8 L) (n = 12 patients) for 20 mg/day and 22.2 L (±19.7L) (n=5) for 40 mg/day.

Elimination

Because of early immune response, pegvaliase is primarily cleared by immune-mediated mechanisms. In study 165-302 Part 3 (Week 62), clearance of pegvaliase (PFS) stabilized and was eliminated with a mean (SD) half- life of 47.3 hours (41.6 hours) and 60.2 hours (44.6 hours) at 20 mg and 40 mg respectively. Individual values for half-life ranged from 14 to 132 hours.

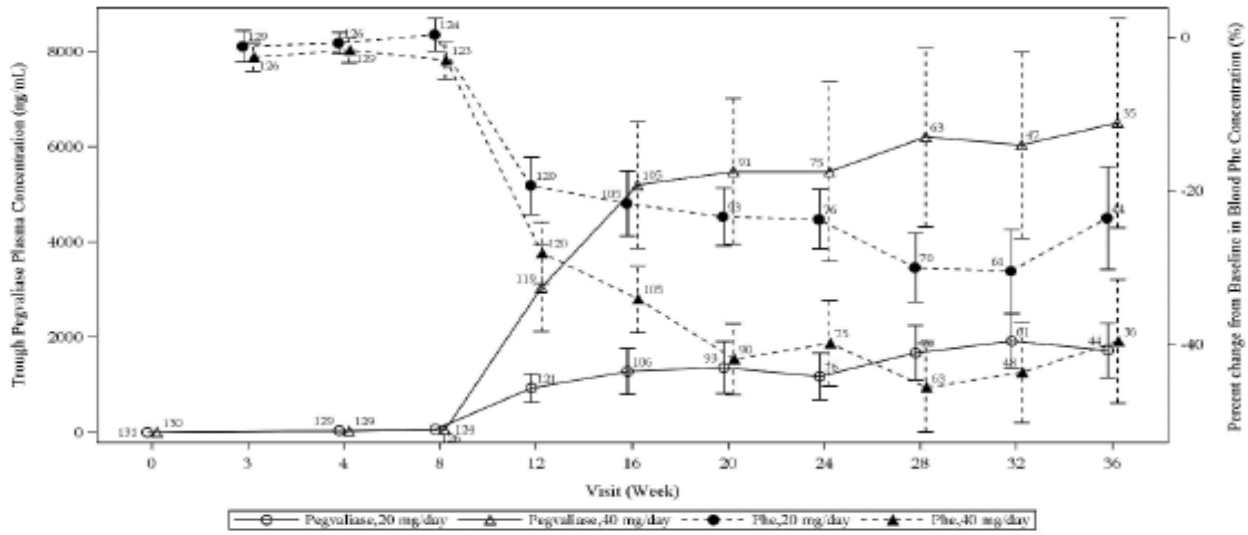
In study 165-302, the apparent clearance was also different between the dose level of 20 mg and 40 mg daily for both VS and PFS after Week 57. With PFS in Week 62, the CL_{ss}/F with 20 mg daily was 0.39 L/h, whereas with 40 mg daily the CL_{ss}/F was 1.25 L/h.

Dose proportionality and time dependencies

- **Dose proportionality**

Based on study 165-301, the pegvaliase Ctrough concentrations over the dose range of 20 and 40 mg/day showed a more than dose proportional increase (**Figure 7**).

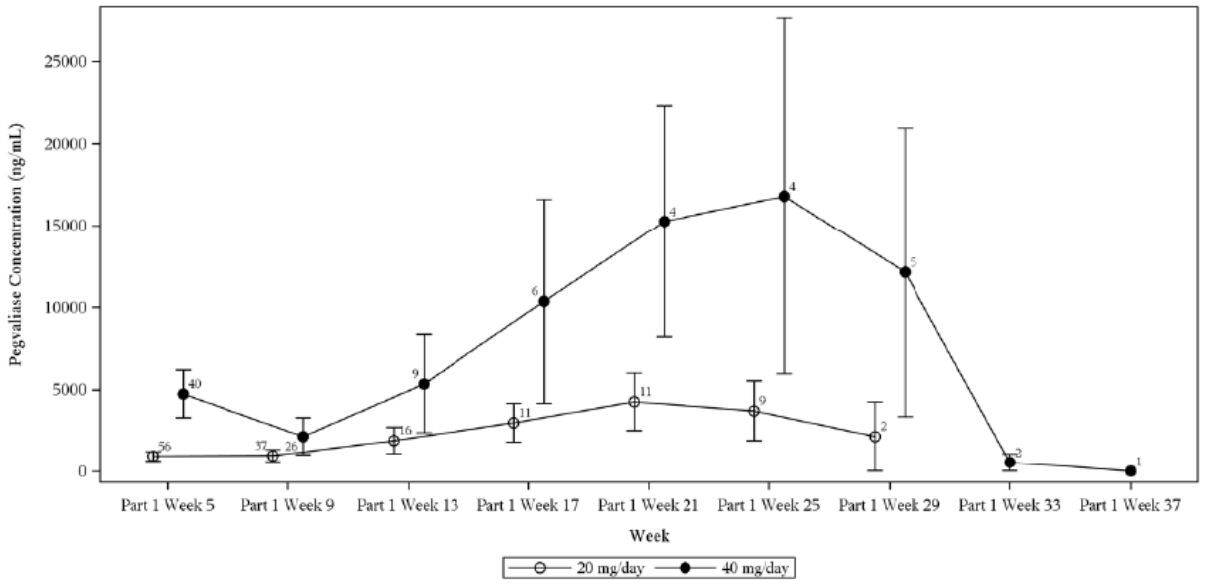
Figure 6. Mean trough Pegvaliase concentration over time by randomised dose group (Study 165-301)



PK, pharmacokinetics
Error bars represent standard error.
All measured pegvaliase concentrations (collected pre-dose or postdose) were treated as trough concentrations.

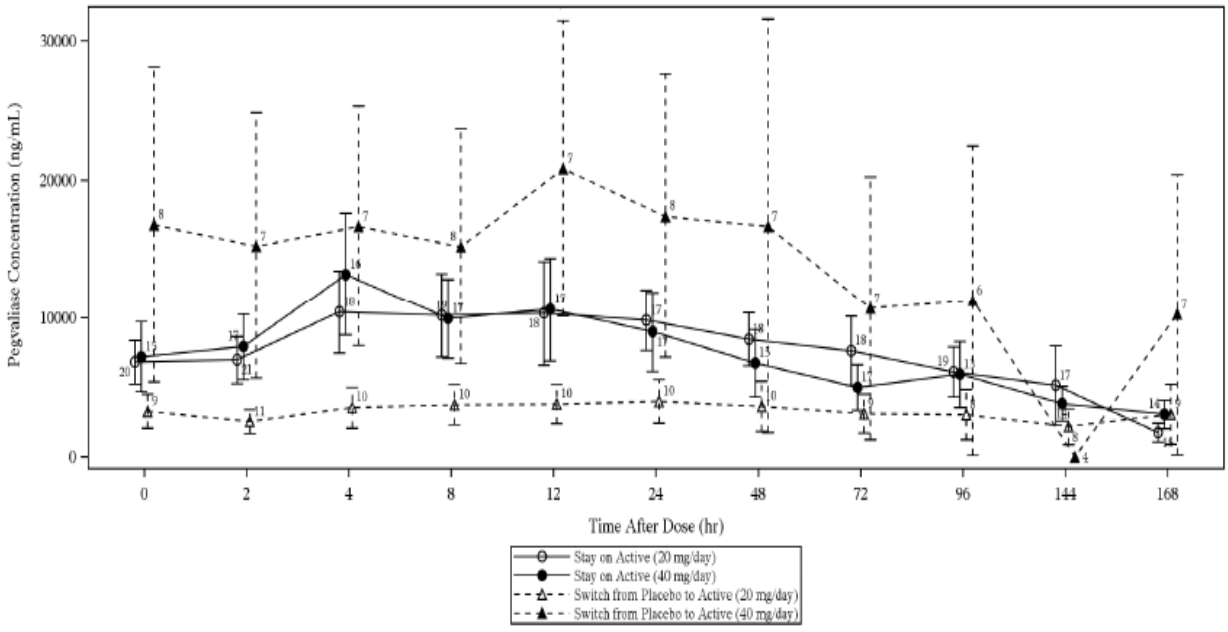
However, in study 165-302, the exposure between at the 20 mg/day and 40 mg/day dose is much less than dose proportional for parts 1 and 2(**Figure 7** part 1, part 2 not shown).

Figure 7. Mean (SE) Plot of Trough Pegvaliase Concentration Over Time Analysis Population: All Subjects Entered 165-302 (165-302 Part 1 Data)



In Part 3 of the study, the mean Ctrough pegvaliase is comparable between the 20 mg/day and 40 mg/day group (**Figure 8**).

Figure 8. Mean (± SE) Concentration Profiles of Pegvaliase at Part 3 Week 5/6 by Dose Group (Study 165-302 Part 3)



- **Time dependency**

The dosing paradigm in phase III was changed to an Induction/Titration (I/T) regimen based on tolerability in the phase 2 studies. In Induction/Titration (I/T) regimen, apparent clearance of pegvaliase was increased in many subjects and plasma exposure was low to non-detectable after Week 1-2 due to

the early immune response. Later after the antibodies level stable, the concentration increased. In phase 3 studies at in the maintenance phase steady state was observed between 4 – 24 weeks.

- **Pharmacokinetics in target population**

All the clinical studies were conducted in phenylketonuria (PKU) patients. Different regimens have been used in Phase 2 and Phase 3 studies. The dose regimens proposed in the SmPC are used in Phase III studies, so only results from those studies are presented in this report.

Patients started with a starting regimen (weekly dose), then titration regimen in study 301, followed up in study 302 in which all patients entered maintenance regimen treated with daily dose of pegvaliase. With the 2.5 mg weekly starting dose regimen, the pegvaliase plasma concentration was very low or below the LLOQ after Week 1-2. PK parameters of pegvaliase were only calculated based on the PK samples from Week 1 (VS) or Week 5/6 (PFS) in Part 3 of study 302 **Table 13**, concentration time curves for PFS in **Figure 7**), which is around 60-70 weeks after the starting regimen (study 165-301)

Table 13. Pegvaliase Pharmacokinetic Parameters at Part 3 Week 1 and Week 5/6 by Dose Group in Study 165-302

Parameter	Statistic	Vial and Syringe (Week 1)		Prefilled Syringe (Week 5/6)			
		Stayed on Active		Stayed on Active		Switched from Placebo to Active	
		20 mg/day n=19	40 mg/day n=14	20 mg/day n=20	40 mg/day n=18	20 mg/day n=11	40 mg/day n=8
$AUC_{0\text{-}24hr,ss}$ (ng*hr/mL)	n	12	7	17	12	7	7
	Mean	143039.7	206572.3	262181.5	246782.5	116564.8	454920.8
	SD	174109.69	212342.03	280378.19	338587.54	111571.12	649658.74
	Median	75500.3	109050.2	210350.9	109603.2	89065.4	30964.7
	Min, Max	16058 , 620176	18761 , 520775	5470 , 1126030	4507 , 1169209	10583 , 319763	5482 , 1425093
$C_{max,ss}$ (ng/mL)	n	16	10	17	15	8	8
	Mean	7098.4	10653.4	14042.6	16687.3	5334.5	23053.8
	SD	7971.62	13438.66	16254.69	19457.12	5209.85	35974.26
	Median	4535.0	6930.0	11400.0	8090.0	3435.0	1118.0
	Min, Max	824 , 32300	954 , 44100	255 , 68500	239 , 63800	536 , 15500	91 , 91500
$C_{trough,ss}$ (ng/mL) [a]	n	14	8	15	14	8	8
	Mean	6464.8	9727.3	11177.3	10446.0	4995.9	17408.5
	SD	6841.81	10438.97	8974.65	12723.89	5216.14	29014.29
	Median	4420.0	5035.0	11400.0	5265.0	2845.0	833.5
	Min, Max	647 , 25900	688 , 28500	210 , 29600	176 , 43100	347 , 15500	0 , 66100
$C_{avg,ss}$ (ng/mL)	n	12	7	17	12	7	7
	Mean	5960.0	8607.2	10924.2	10282.6	4856.9	18955.0
	SD	7254.57	8847.58	11682.42	14107.81	4648.80	27069.11
	Median	3145.8	4543.8	8764.6	4566.8	3711.1	1290.2
	Min, Max	669 , 25841	782 , 21699	228 , 46918	188 , 48717	441 , 13323	228 , 59379
$T_{max,ss}$ (hours)	n	16	10	17	15	8	8
	Mean	9.8	10.9	9.8	7.5	16.6	8.4
	SD	8.07	9.64	8.06	4.64	7.39	7.83
	Median	8.2	7.9	8.0	8.2	16.5	8.0

Parameter	Statistic	Vial and Syringe (Week 1)		Prefilled Syringe (Week 5/6)			
		Stayed on Active		Stayed on Active		Switched from Placebo to Active	
		20 mg/day n=19	40 mg/day n=14	20 mg/day n=20	40 mg/day n=18	20 mg/day n=11	40 mg/day n=8
	Min, Max	0 , 24	0 , 24	0 , 24	0 , 12	8 , 24	0 , 24
$t_{1/2}$[b] (hours)	n	NA	NA	13	8	5	3
	Mean	NA	NA	47.3	60.2	49.6	26.5
	SD	NA	NA	41.56	44.59	37.94	11.85
	Median	NA	NA	26.9	56.1	39.1	28.6
	Min, Max	NA	NA	14 , 132	14 , 127	15 , 115	14 , 37
CL_{ss}/F (mL/hr)	n	12	7	17	12	7	7
	Mean	384.1	657.6	394.4	1246.3	487.9	2509.0
	SD	365.11	763.33	866.36	2457.93	638.88	3012.89
	Median	267.2	366.8	95.1	406.2	224.6	1291.8
	Min, Max	32 , 1246	77 , 2132	18 , 3657	34 , 8876	63 , 1890	28 , 7296
Vdz/F[b] (mL)	n	NA	NA	13	5	4	3
	Mean	NA	NA	26448.3	22240.3	34518.3	72949.5
	SD	NA	NA	64806.23	19706.48	43550.85	70141.70
	Median	NA	NA	5997.8	20627.0	14259.4	69298.7
	Min, Max	NA	NA	1798 , 240924	3126 , 49487	9797 , 99757	4705 , 144845

$AUC_{0\text{-}24hours}$, area under the plasma drug concentration time curve to 24 hours postdose; $C_{avg,\ ss}$, average plasma concentration; CL_{ss}/F, apparent clearance; $C_{max,\ ss}$, maximum plasma concentration; $C_{trough,\ ss}$, plasma trough concentration; NA, not available; PD, pharmacodynamics; PK, pharmacokinetics; SD, standard deviation; ss, steady state; $t_{1/2}$, terminal half-life; t_{max}, time after administration of a drug when the maximum plasma concentration is reached; Vdz/F, apparent volume of distribution.

[a] The concentration values measured within 24±2 hours post-dose were reported as Ctrough,ss for better accuracy.

[b] Values of $t_{1/2}$ and Vdz/F were not reported for the intensive PK/PD visit of Part 3A because the terminal phase was not well characterized due to short sampling duration (24 hours).

Special populations

No dedicated studies were conducted to investigate the impact of impaired renal or hepatic functions. Patients included in all studies had normal hepatic and renal function.

The effect of race could not be investigated due to the study population (97% Caucasians). Subjects aged above 65 years were not included in the clinical studies.

Pharmacokinetic interaction studies

No pharmacokinetic interaction studies were submitted.

2.4.3. Pharmacodynamics

Mechanism of action

Pegvaliase is a pegylated phenylalanine ammonia lyase enzyme that converts phenylalanine to ammonia and trans-cinnamic acid that are metabolized by the liver and excreted in the urine, respectively. Pegvaliase reduces blood phenylalanine levels and as such substitutes for the deficient PAH enzyme. Contrary to PAH which acts intracellularly and is modulated by cofactor tetrahydrobiopterin (BH4) pegvaliase acts in the blood compartment independently from BH4.

Primary and Secondary pharmacology

Reduction of blood phenylalanine levels was either chosen as primary (part 2 of study 165-302) or secondary endpoint in clinical studies of this dossier. Data on the reduction of blood phenylalanine levels are discussed in the clinical efficacy paragraph of this report.

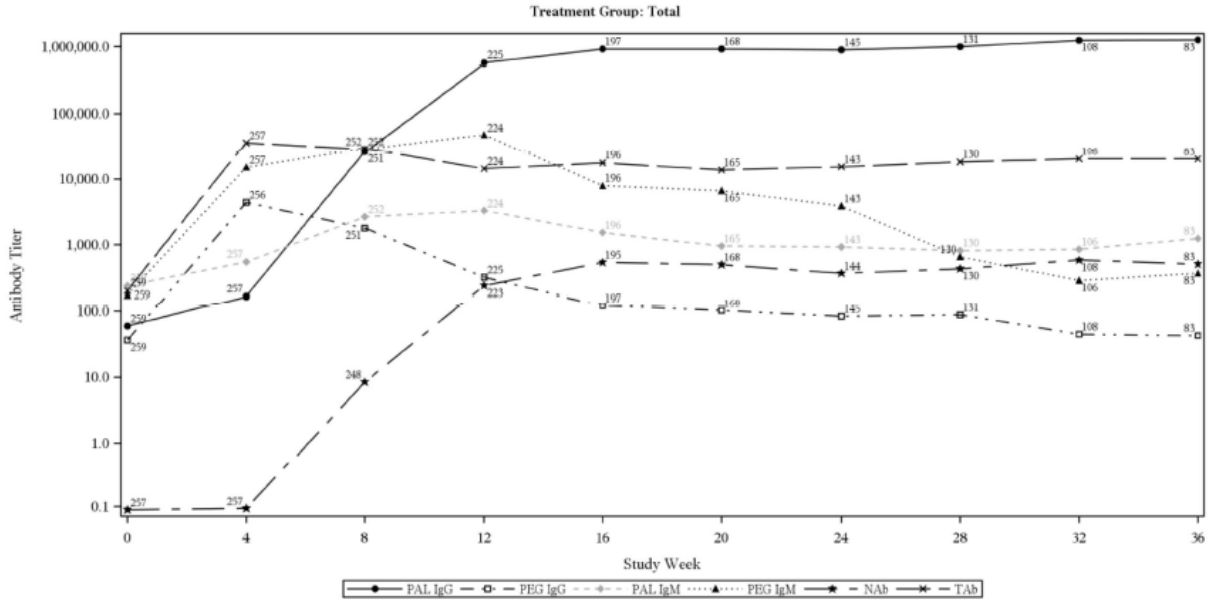
No secondary pharmacology studies were submitted, as off-targets of pegvaliase are not expected.

Immunogenicity

Pegvaliase immunogenicity was monitored using a panel of semi-quantitative immunoassays to detect antibodies directed against the total drug (TAb) and specific isotypes against each component of the drug (PAL IgM, PAL IgG, PEG IgM, and PEG IgG). NAb capable of inhibiting enzymatic activity of the PAL enzyme were also measured. IgE antibodies specific to PEG-PAL or PAL alone were monitored using assays yielding a positive or negative result. Blood samples for routine immunogenicity testing were collected prior to dosing.

Antibody titre over time is presented for study 165-301 below (**Figure 9**).

Figure 9. Antibody titers over time in log-scale plot (Study 165-301 all patients).



IgG, immunoglobulin G; IgM, immunoglobulin M; TAb, total antibody; NAb, neutralizing antibodies; PAL, phenylalanine; PEG, polyethylene glycol.
Antibody titer is the mean titer for each antibody type at each timepoint.

The number of subjects in the different populations in Study 165-302 that developed ADA's are presented in **Table 14** and the reduction of phenylalanine against the observed NAbs titres in **Figure 10**.

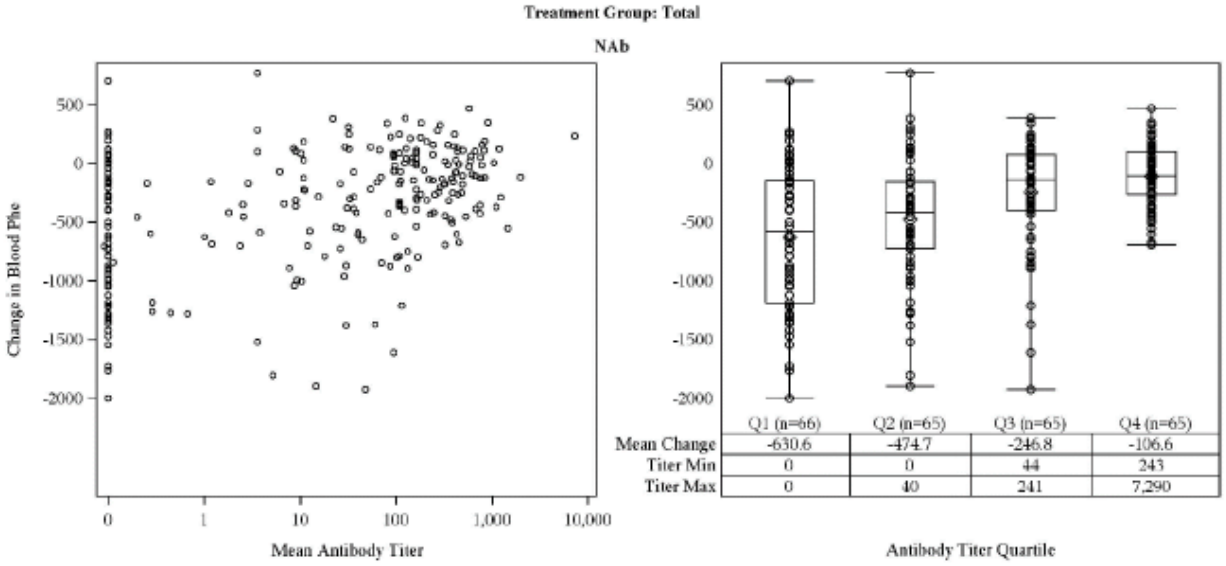
Table 14. Incidence of NAb Positivity and Mean Titres in Part 1 and Part 4 in Study 165-302

	Analysis Population	**Part 1**	**Part 4**		
		Week 1	**Week 1**	**Week 41**	**Week 73**
Total number of patients	Safety	162	184	171	91
RDT	76	68	64	45	
Non-RDT	40	66	61	21	
Percent Positive	Safety	76.5%	78.8%	80.1%	76.9%
RDT	59.2%	79.4%	76.6%	77.8%	

Non-RDT	97.5%	92.4%	96.7%	100%	
Mean/Median Titer (Range)	Safety	456/162 (0 – 4,374)	611/162 (0 – 13,122)	581/162 (0 – 13,122)	442/162 (0 – 4,374)
RDT	321/36 (0 – 4,374)	313/162 (0 – 1,458)	435/162 (0 – 4,374)	511/54 (0 – 4,374)	
Non-RDT	751/486 (0 – 4,374)	1,129/486 (0 – 13,122)	938/486 (0 – 13,122)	429/486 (2 – 1,458)	

RDT: Randomised withdrawal. The RDT Population was defined as all subjects in the mITT Population who were originally enrolled in 165-301, were randomized into Part 2, and entered Part 4; The Non-RDT Population was defined as all subjects who entered Part 4, were originally enrolled in 165-301, and did not participate in Part 2 because they did not have ≥20% blood phenylalanine reduction. The Non-RDT Population was a subset of the population of subjects who did not participate in Part 2 who did not have blood phenylalanine reduction of ≥ 20% from naïve baseline prior to entering Part 4.

Figure 10. Change in blood phenylalanine concentration and Mean NAb Titre (All patients, Study 165-302)



NAb, neutralizing antibodies
Change in blood phenylalanine concentration was from Baseline to end of study for each patient. Each dot represents1 patient.
In the right panel, patients were divided into quartiles based on their mean NAb titre. The horizontal lines from top to bottom represent the maximum value.

2.4.4. Discussion on clinical pharmacology

During the clinical development program, pegvaliase from four different processes was used (A-D), in which bioequivalence has been shown between processes A and the higher PEG containing process B, indicating that increasing the number of PEG does not improve the exposure of pegvaliase. Pegvaliase from process C and D contain the same number of PEG as from Process A. Only pegvaliase from process D was used in Phase III studies, and will be used for commercial formulation as PFS.

There was high inter-individual variability with the PFS form of the product. Different injection sites or the number of injections had no impact on the PK. Although the variation in C_{trough} level cannot be explained, considering the close monitoring for phenylalanine level in patients in practice, and that the dose will be

adjusted based on response, the variation in C_{trough} from the clinical trials is not considered clinically relevant.

The absolute bioavailability of pegvaliase with subcutaneous injection in humans remains unknown. However, this is acceptable as overall the pharmacokinetics appear to be highly individualised and dependent on the individual's immune response.

Following a single dose subcutaneous injection, pegvaliase is slowly absorbed and reaches maximum concentration in plasma around 4-5 days after administration. With the 2.5 mg weekly starting dose regimen, the pegvaliase plasma concentration was very low or below the LLOQ after Week 1-2. This is because immune-mediated clearance starts early (including baseline antibodies) and plays a major role in the elimination of pegvaliase. Because the total antidrug antibody level (ADA) reaches a maximum around Week 4 (study 165-301) and then becomes stable in Week 4-12, the pegvaliase plasma concentration increases after Week 3-4. Then the concentration continued to increase in the titration dose regimen along with the increase of the dosage.

In the maintenance regimen, a daily dose of 20, 40 or 60 mg was given and, because of the slow absorption and frequent daily dose scheme, the steady state of pegvaliase C_{trough} level can be reached in 4-24 weeks in maintenance treatment in most of the patients.

PK parameters (such as Vz/F, CL/F, $t_{1/2}$, C_{max} and AUC_{24h}) are calculated based on the pegvaliase concentrations with PFS from Part 3 of phase III study 165-302 (i.e. Week 62 after starting the treatment). Due to the study design (i.e. inclusion criterion of >20% Phe reduction, injection sites, and the number of injections), the PK data should be interpreted cautiously. Vz/F is 26.4 L (±64.8 L) (n=12 patients) for the 20 mg/day and 22.2 L (±19.7L) (n=5) for the 40 mg/day dose. The CL_{ss}/F with 20 mg daily is 0.39 L/h, whereas with 40 mg daily the CL_{ss}/F is 1.25 L/h. The mean (SD) half-life is 47.3 hours (41.6 hours) and 60.2 hours (44.6 hours) at the 20 mg and 40 mg daily dose, respectively. It should be noted that in part 3 of study 165-302, pegvaliase C_{trough} levels between 20 mg and 40 mg daily dose with PFS are comparable. This was due to the inclusion criterion (i.e. >20% reduction of phenylalanine) applied in part 2 of the study, so only the patients who have relatively high C_{trough} were included in further treatment. Furthermore, patients were not randomised, and the dose was chosen based on the response of patients.

Because of immune-mediated clearance, the inter-individual variability in pegvaliase exposure was high (142 – 301%).

No patients with renal or hepatic impairment were included in the clinical studies. This was considered acceptable, because proteins are primarily cleared by catabolism and therefore no direct effect of hepatic/renal function on the pharmacokinetics of pegvaliase is expected. Nevertheless, the impact of the elimination of PEG, trans-cinnamic acid and ammonia (substrate after catabolism of phenylalanine) in renal/hepatic impaired patients has been included in the RMP as missing information.

Anti-drug- antibodies (ADA) were detected in all patients treated with pegvaliase and independently from the dose levels. Patients with positive baseline of neutralising antibodies (NAb) had a higher incidence of not achieving the 120 – 600 µmol/L phenylalanine in the studies. However, as in practice the dose will be adjusted based on the individual phenylalanine level rather than the fixed dose in the studies, clinical consequences due to positive baseline NAb are not expected. In addition, considering the hypersensitivity issue in the patients, it is recommended that the applicant develops an assay to measure anti-PEG IgE and submit the final study report. Furthermore, anti-PEG antibody titers were high during induction and titration phase but decreased to low titers in maintenance phase. Therefore, anti-PEG antibodies may have interfered with PK measurements during the induction and titration phases. As pegvaliase dosing was determined on patient tolerability and blood phenylalanine reduction, this had no clinical impact.

Overall, in all clinical studies an inverse relationship between ADA response and pegvaliase plasma exposure was observed suggesting that subjects who mount a stronger immune response to pegvaliase are likely to have a lower pegvaliase plasma exposure. The inverse relationship between antibody titers and pegvaliase trough concentrations appeared to be present for all isotopes of anti-drug antibodies.

In Part 1 of study 165-302, while patients were still on doses of 20 or 40 mg/day pegvaliase, patients with lower mean antibody titres achieved the largest reductions in blood phenylalanine concentration. In Part 4, mean blood phenylalanine concentrations continued to decrease for all patients including those in the higher antibody titre quartiles (Q3 and Q4). This at least is reassuring in so far that patients with high neutralising antibodies still show efficacy in terms of phenylalanine reduction, and that dose increases do not induce an increase in antibody titres. It is however uncertain what the impact of high NAb titers is in patients on long term pegvaliase treatment. Immunogenicity data on long term pegvaliase treatment from study PAL-003 and 165-302 part 4 is expected in the final study reports. Immunogenicity and antibody response are addressed with appropriate warnings in the SmPC.

A clear PD effect of pegvaliase was demonstrated in the pivotal part 2 of study 165-302 (placebo-controlled withdrawal phase). The PD effect was further supported by data in the long-term studies PAL-003 and 165-302 part 4.
Because antibody-mediated clearance is the primary mechanism by which the drug is cleared from plasma, patients with higher antibody titres required higher doses of pegvaliase to reach sustained blood phenylalanine reduction. As doses were optimized (adjusted for blood phenylalanine reduction and safety) in Part 4 of study 165-302, mean blood phenylalanine concentrations continued to decrease in all Part 4 analysis populations (Part 4 Safety Population, RDT Population, and Non-RDT Population) despite sustained anti-drug antibody titres.

Given the observation that patients in the non-RDT population did not show a decrease in phenylalanine levels in the titration phase after desensitisation may be explained by the higher observed antibody titres in these patients (Figure 12). Nevertheless, in part 4 of study 165-302, meaningful phenylalanine reductions were also noted in the non-RDT patients after a dose increase.

2.4.5. Conclusions on clinical pharmacology

The available pharmacokinetic data even though limited are considered sufficient, given that response to treatment is highly individualised and the product is titrated based on response.

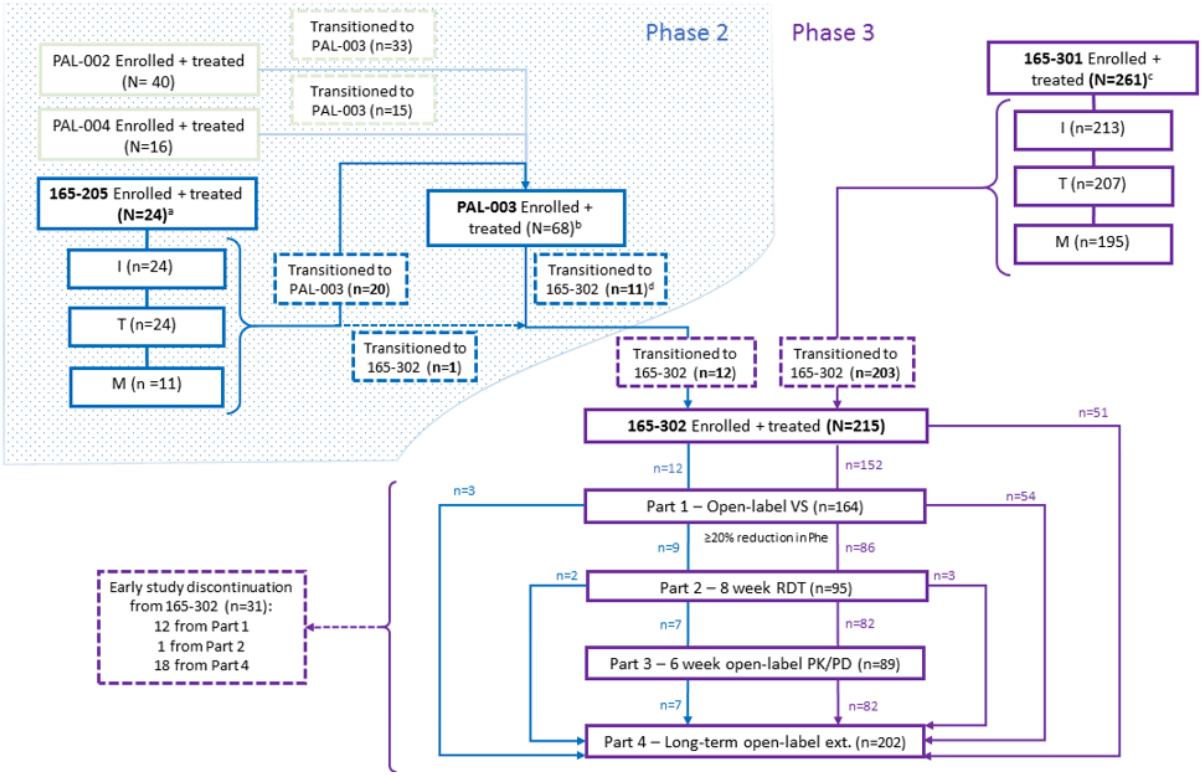
A sustained pharmacodynamic effect of pegvaliase in reducing baseline phenylalanine in PKU patients has been demonstrated. It is recommended that the applicant develops a new format for measurement of anti-PEG IgE in human serum using electrochemiluminescence to further characterise the role of antiPEG antibodies in hypersensitivity reactions to pegvaliase treatment.

2.5. Clinical efficacy

The proposed indication is supported by data from seven clinical studies: a single-dose Phase 1 study (PAL-001); 3 multi-dose Phase 2 studies (PAL-002, PAL-004, and 165-205) that were followed by the Phase 2 long-term open-label extension study (PAL 003); and two Phase 3 studies (165-301 and 165-302), and the 165-302 sub-study (165-303).

Figure 11 provides a summary of all the clinical studies including patient disposition.

Figure 11. Patient Disposition across the Phase II/Phase III with pegvaliase



The I/T/M Population (N=285) included 24 patients from 165-205 (including those who transitioned to PAL-003 and 165-302) + 261 patients from 165-301 (including those who transitioned to 165-302).
a 165-205: 1 patient completed the study and 2 stopped treatment early due to an AE; 21 patients transferred to another study, 20 to PAL-003 and 1 to 165-302 (N=24).
b PAL-003: 37 patients are ongoing in the study and 20 discontinued from the study; 11 patients transitioned to 165-302 (N=68).
c 165-301: 4 patients completed the study without entering into 165-302, 48 patients discontinued from the study and a further 6 discontinued from study drug; 203 transitioned to 165-302 (N=261).
d 3 Patients started pegvaliase in PAL-002, enrolled into PAL-003 and transferred to 165-302 extension; 5 Patients started pegvaliase in PAL-004, enrolled into PAL-003 and transferred to165-302 extension.; 3 Patients started pegvaliase in 165-205, enrolled into PAL-003 and transferred to 165-302 extension.

2.5.1. Dose response studies

Study PAL-002 was a 16 weeks open-label, dose-finding study to evaluate the safety, efficacy, and tolerability of multiple subcutaneous doses of rAvPAL-PEG in patients with PKU. The study consisted of two parts. In part 1, patients received a fixed pegvaliase dose for 8 weeks (0.001 mg/kg/week to 0.1 mg/kg/week). In part 2, the 8 weeks induction period was followed by an additional 8 weeks of adjustable dosing (up to 5 mg/kg/week) to reach a blood phenylalanine levels of 60-600 µmol/L. Study PAL-002 enrolled 40 patients of whom 11 patients were previously treated with pegvaliase in study PAL-001 (see pharmacokinetics section).

Study PAL-002 did not demonstrate a clear pharmacological nor any clinical relevant effect on the phenylalanine reduction.

Study PAL-004 was a phase II, 16 weeks, multi-centre, open-label study to evaluate the safety, tolerability, and efficacy of subcutaneous dose levels of pegvaliase administered daily in patients with PKU. Study PAL-004 included 16 PKU patients all receiving starting doses based on bodyweight. Patients received pegvaliase 0.06 mg/kg/day, 0.1 mg/kg/day, 0.2 mg/kg/day, 0.4 mg/kg/day, 0.6 mg/kg/day, or 0.8 mg/kg/day for 5 days per week for 13 weeks, followed by a 3 weeks safety assessment follow-up without pegvaliase dosing. The enrolment started with the 0.4 mg/kg/day dose and patients could be

titrated up or down depending on the tolerability and/or efficacy. The pharmaceutical product used in the study was vial+syringe (VS).

The mean weekly dose was 0.486 mg/kg (range, 0.09 mg/kg to 1.14 mg/kg). At week 2 of the study a phenylalanine level ≤600 µmol/L was observed in all 16 patients, however this effect could not be sustained throughout the rest of the study. Only 4 patients had measurable drug concentrations across multiple study visits. Exposure decreased after week 2.

Study 165-205

Study 165-205 was a phase II, multi-centre, open-label, dose-finding study to evaluate safety, efficacy, and tolerability of subcutaneously administered pegvaliase in patients with PKU.

This 25-week study included 24 weeks of pegvaliase administration followed by a 1 week of post-treatment safety follow-up. Twenty-four patients, naïve to pegvaliase treatment, were enrolled. The study was designed to find the optimal therapeutic dose, guided on phenylalanine levels (target is phenylalanine levels 60 to ≤600 µmol/L) and by safety/tolerability.

Pegvaliase administration was initiated with an induction period, followed by a titration period and a maintenance period. Dosing was initiated as a fixed sub-therapeutic dose of 2.5 mg/week (induction period) to expose patients to the immunogenic properties of pegvaliase. Following the induction period, dose was titrated upwards (to a maximum weekly dose of 75 mg/day [375 mg/week]) towards the goal of reducing blood phenylalanine to a target of ≤600 µmol/L while minimizing the onset of hypersensitivity AEs. A therapeutic dose was reached once a blood phenylalanine reduction of ≤600 µmol/L was achieved and sustained for a minimum of 4 weeks (primary endpoint). The pharmaceutical product used in the study was the VS product.

After a 4 weeks (mean 3.1 (±0.66) weeks) induction phase with 2.5 mg/week of pegvaliase, patients were titrated up to an effective target dose. Eleven of 24 (46%) patients achieved the target sustained phenylalanine levels ≤600 µmol/L for at least 4 weeks. Nine of these 11 patients maintained phenylalanine ≤600 µmol/L from week 12 to the end of the study week 24. For the patients who achieved the target dose, the average amount of weekly pegvaliase was 64.7 mg/week (SD, 45.52). For those patients who did not achieve target dose the average amount of pegvaliase was 89.4 mg/week (SD, 74.88). By the end of the 24-week study drug administration period, median blood phenylalanine levels in the overall population were reduced to 644.0 µmol/L (median), a mean decrease of -551.2 µmol/L (SD, 558.38) from baseline and -46.2 % change from baseline (SD, 42.77).

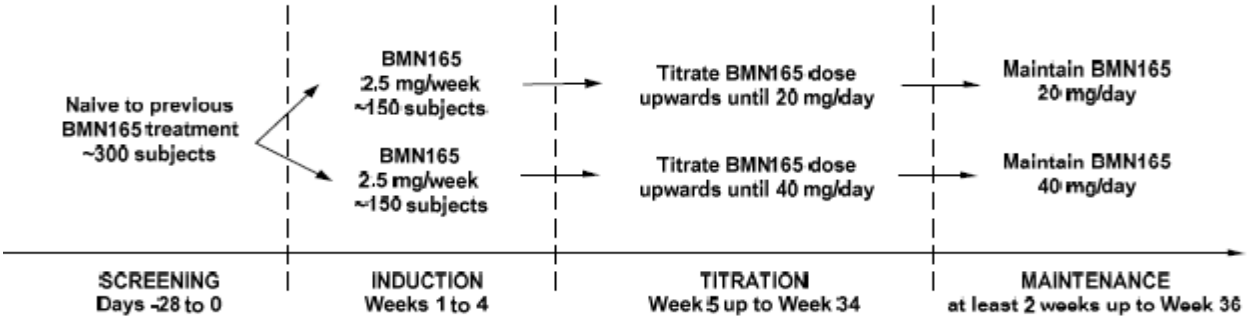
At the end of study 165-205, 20 patients were transitioned to study PAL-003. One patient was transitioned to study 165-302.

Study 165-301

S**tudy 165-301**: a four-part, phase III, randomized, double-blind, placebo-controlled, four-arm, discontinuation study to evaluate the efficacy and safety of subcutaneous injections of pegvaliase self-administered by adults with PKU. The study consisted of an induction, titration, and maintenance regimen.

Figure 12 provides a schematic of the overall study design.

Figure 12. 165-301 Study Design



PD, pharmacodynamics; PK, pharmacokinetic.
a During Part 4, open-label pegvaliase may be administered at a dose of 5, 10, 20, 40, or 60 mg/day.

Initially study 165-301 enrolled patients aged ≥16 to ≤70 years of age. As a safety precaution the protocol was amended (amendment 2; 18 August 2014) to only allow enrolment of patients ≥18 years to ≤70 years old. PKU patients with phenylalanine levels >600 µmol/L at baseline and phenylalanine levels >600 µmol/L for the 6 months prior to screening could be enrolled in the study. The paediatric patients aged 16 years to 18 years already enrolled could continue the treatment in the study and they could also participate in study 165-302. In total 261 patients were enrolled, 131 patients were randomised to a target dose of 20 mg/day and 130 patients to 40 mg/day.

Of the 261 patients enrolled 196 (75.1%) patients were previously treated with sapropterin. Twenty-eight (28) patients were considered treatment naïve (10.7%). The majority (144/196; 73.5%) of the sapropterin treated patients were considered sapropterin non-responders. Fifty-two (52) of 196 patients were regarded as sapropterin responders. The treatment responses were not originally collected for 37 patients as per the original protocol. However, using previous medication data 6 patients were identified as receiving sapropterin prior to starting 165-301. These 6 patients were considered sapropterin non-responders.

All patients received a fixed starting dose of 2.5 mg/week pegvaliase for 4 weeks, i.e., the induction phase (to desensitise the patients to pegvaliase). After desensitisation patients were titrated toward the assigned randomised final study dose (20 or 40 mg/day). The induction and titration scheme were in line with the scheme used in phase II study 165-205 (see above).

Inclusion and exclusion criteria, objectives and outcomes of the study were the same as in Study 165-302 (see below).

About 57% of the patients depended on MNT as a protein source. Baseline mean (SD) protein from intact food (gram; g) was 39.1 (27.3) in the 20 mg/day and 37.9 (28.3) in the 40 mg/day group.

All 261 subjects enrolled in the study received at least one dose of pegvaliase and were included in the ITT and safety populations.

The primary objective of study 165-301 was to characterize the safety and tolerability in pegvaliase-naïve patients who self-administered pegvaliase up to fixed maintenance dose levels of 20 mg/day or 40 mg/day. The secondary objective of the study was to evaluate blood phenylalanine concentration during induction, titration, and maintenance. The pegvaliase dose (e.g. 2.5 mg/week) used in the induction phase is too low to expect an efficacy response. Therefore, the induction phase will not be further discussed with regard to efficacy.

Treatment with pegvaliase reduced mean blood phenylalanine concentration for both the 20 mg/day and the 40 mg/day randomized dose groups. The median reduction from baseline for all patients (ITT population) was -363.0 µmol/L at week 28 (n = 133) and -191.5 µmol/L at Week 36 (n = 80). There was a statistically significant difference (p-value=0.0269) between the randomized dose groups favouring the 40 mg/day group. The least squares mean of the difference between the 20 mg/day group and 40 mg/day group was -112.8 µmol/L (95% confidence interval: -212.5, -13.1 µmol/L). A higher percentage of patients in the 40 mg/day randomized dose group compared to the 20 mg/day randomized dose group achieved blood phenylalanine reductions to ≤600 µmol/L (61/130 [46.9%] *versus* 45/131 [34.4%]). Median time to reach phenylalanine levels ≤600 µmol/L was 78.0 [14, 218] days for 20 mg/day and 78.0 [15, 192] days in the 40 mg/day group.

Of the 261 enrolled patients, 195 (75%) patients reached their randomised maintenance dosage (103 patients in the 20 mg once daily arm, 92 patients in the 40 mg once daily arm). Patients in the 20 mg once daily randomised arm reached their maintenance dosage at a median time of 10 weeks (range: 9 to 29 weeks) and patients in the 40 mg once daily arm reached their maintenance dosage at a median time of 11 weeks (range: 10 to 33 weeks). Overall 66/261 patients (25.3%) did not reach the maintenance phase of the study. Twenty-four of the 66 patients did not reach the randomised dose due to early study closure (discussed below). The median time to reach the maintenance period was 10.1 weeks in the 20 mg/day randomized dose group (103 patients), 11.1 weeks in the 40 mg/day randomized dose group (92 patients). It should be noted that the selection of patients who can reach the maintenance phase are the patients who can tolerate the drug.

Of the 261 patients in Study 301, 11 patients were aged between 16 and 18 years at enrolment. All 11 patients had inadequate blood phenylalanine control (blood phenylalanine levels above 600 µmol/l) at baseline. These patients received the same induction/titration/maintenance regimen as patients aged 18 years and older in this study. Mean (SD) change from baseline was 20 (323) µmol/l at Month 12 (n=9), 460 (685) µmol/l at Month 24 (n=5), and 783 (406) µmol/l at Month 36 (n=5). Of the 11 patients initially enrolled in Study 301, 3 patients reached blood phenylalanine levels ≤ 600 µmol/l by 12 months, 7 patients reached this threshold by 24 months, and 8 patients reached this threshold by 36 months.

In the Phase 3 pegvaliase program, Study 165-301 served as a feeder study designed to select an appropriate subject population for evaluating the efficacy of pegvaliase in the placebo-controlled part of 165-302. Once recruitment for 165-302, Part 2 was completed, 165-301 was closed early as the primary study objective of identifying subjects for the randomized discontinuation trial had been fulfilled.

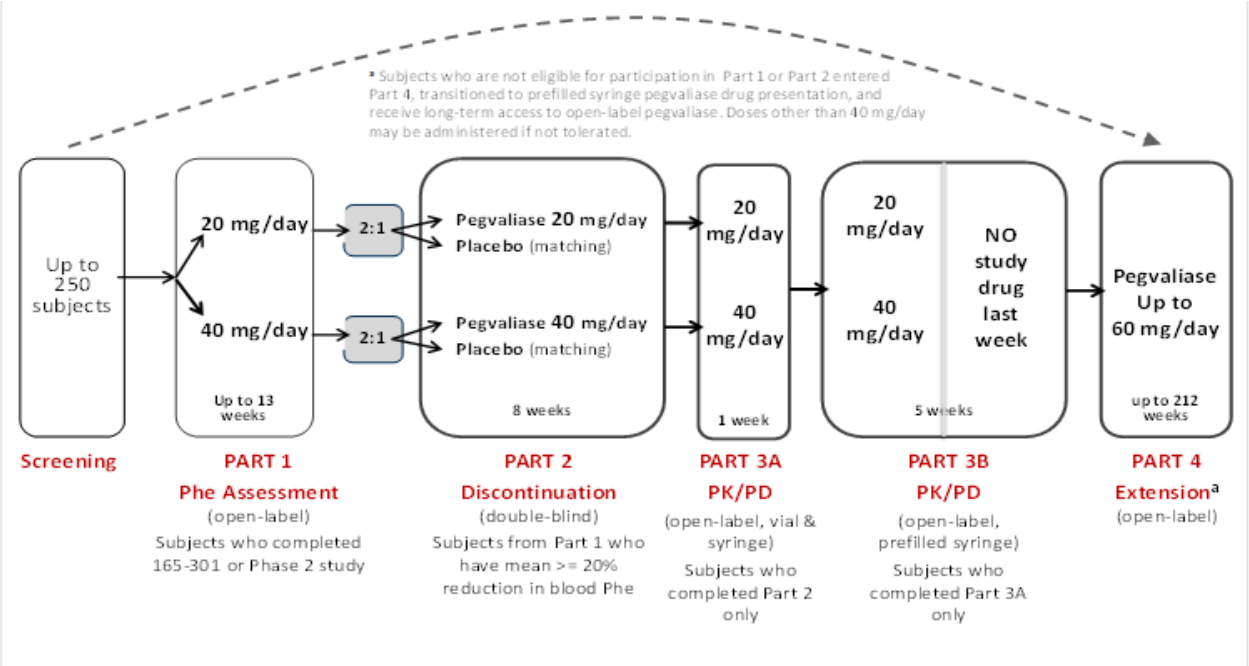
2.5.2. Main study

Study 165-302: a four-part, phase 3, randomized, double-blind, placebo-controlled, four-arm, discontinuation study to evaluate the efficacy and safety of subcutaneous injections of pegvaliase self-administered by adults with phenylketonuria.

Methods

Figure 13 provides a schematic of the overall study design.

Figure 13. 165-302 Study Design



Study Participants

Inclusion criteria (selection)

Individuals had to meet the following criteria to be eligible to participate in this study:

- Completed a prior pegvaliase study (PAL-003, 165-205, or 165-301) prior to screening.
- Had a stable pegvaliase dose regimen for at least 14 days prior to screening.
- Were at least 18 years of age and no older than 70 years of age at time of screening.
    - Subjects who were 16 to <18 years old could have enrolled into this study under Amendment #1 (10JAN2014).
- Identified a person who was >18 years of age who had the neurocognitive and linguistic capacities to comprehend and complete the POMS (Observer-Rated) scale.
- Identified a competent person or persons who were > 18 years of age who could observe the subject during study drug administration and for a minimum of 1 hour following administration during Part 3, Week 1; Part 4, Week1; if needed upon return to dosing after an AE; if dosing was increased during Part 4; and per investigator determination.
    - A home healthcare nurse could have performed the study drug observations.
- Were willing and able to provide written, signed informed consent after the nature of the study had been explained and prior to any research-related procedures. For minors, a parent or guardian provided written consent and assent could have been requested.
- Received documented approval from a study dietitian confirming that the subject was capable of maintaining their protein intake in accordance with the study protocol.
- Had the neurocognitive and linguistic capacities to comprehend and answer prompts for the ADHD-RS (Investigator-Rated) instrument and to complete the POMS (Subject-Rated) scale.

Exclusion criteria (selection)

Individuals who met any of the following exclusion criteria were not eligible to participate in the study:

- Used any investigational product (except pegvaliase) or investigational medical device within 30 days prior to screening or had a requirement for any investigational agent prior to completion of all scheduled study assessments.
- Used any medication (except pegvaliase) intended to treat PKU, including the use of large neutral amino acids, within 2 days prior to the administration of study drug (Day 1, first dose of pegvaliase).
- Had known hypersensitivity to Dextran or components of Dextran.
- Used or planned to use any injectable drugs containing PEG (except for pegvaliase), including medroxyprogesterone injection, within 3 months prior to screening and during study participation.
- Used levodopa at time of screening.
- Had a positive test for HIV antibody, hepatitis B surface antigen, or hepatitis C antibody (subjects who were not screened in the previous pegvaliase study only).
- Had a history of organ transplantation or was taking chronic immunosuppressive therapy.
- Were participating in the sapropterin registry study (PKU Demographics, Outcomes and Safety [PKUDOS]) at the time of screening.
- Were pregnant or breastfeeding at screening or was planning to become pregnant (self or partner) or breastfeed at any time during the study.
- Had a concurrent disease or condition that could have interfered with study participation or safety (e.g., history or presence of clinically significant cardiovascular, pulmonary, hepatic, renal, hematologic, gastrointestinal, endocrine, immunologic, dermatologic, neurological, oncologic, or psychiatric disease).

Treatments

Study drug (pegvaliase or matching placebo) was administered subcutaneously 7 days/week (i.e., daily) throughout the study. During Parts 1, 3, and 4, subjects were administered open-label pegvaliase. During Part 2, blinded pegvaliase (20 or 40 mg/day) or matching placebo was administered. Pegvaliase VS drug presentation was administered in Parts 1, 2, and 3. Pegvaliase PFS drug presentation was administered starting at Week 2 of Part 3 for subjects who participated in Week 1 of Part 2 or Part 4 for all other subjects.

Objectives

The primary and secondary efficacy objectives of this study were per data from assessments performed during Part 2. PK and PD objectives were per data from Part 3 of this study. Long-term assessments of efficacy were per data from naïve baseline from 165-301 (or the Phase 2 study in which pegvaliase was initiated) through Part 4 of this study (per the data cut-off date).

PART 1: Open-Label Blood Phenylalanine Assessment

The objectives of Part 1 were to screen subjects for eligibility for entry into Part 2 of the study and to characterize the safety of pegvaliase (20 or 40 mg/day) in subjects previously exposed to pegvaliase. There was no efficacy objective for Part 1.

PART 2: Randomized, Placebo-Controlled, Double-Blind Discontinuation

The primary efficacy objective of Part 2 was to evaluate blood phenylalanine concentration in subjects previously exposed to pegvaliase who were administered pegvaliase (20 or 40 mg/day) compared with those who were administered a matching placebo. The secondary efficacy objective of Part 2 was to evaluate inattention and mood symptoms in subjects previously exposed to pegvaliase who were administered pegvaliase (20 or 40 mg/day) compared with those who were administered a matching placebo.

The safety objective of Part 2 was to evaluate the safety of pegvaliase in subjects previously exposed to pegvaliase who were administered pegvaliase (20 or 40 mg/day) compared with those who were administered a matching placebo.

PART 3: Pharmacokinetic/Pharmacodynamic Assessment

The objectives of Part 3 were the following:

- To evaluate multiple-dose PK/PD in subjects who were administered pegvaliase
- To evaluate PK comparability between pegvaliase VS drug presentation and PFS drug presentation

PART 4: Long-Term, Open-Label Extension

The objectives of Part 4 of this study are the following:

- To evaluate the long-term effect of multiple dose levels of pegvaliase on blood phenylalanine concentration in subjects who are administered pegvaliase using PFS drug presentation
- To characterize long-term inattention, hyperactivity, and mood symptoms in subjects who are administered pegvaliase
- To evaluate long-term safety of multiple dose levels of pegvaliase in subjects who are administered pegvaliase using PFS drug presentation
- To characterize protein intake from medical food and intact food in subjects who are administered pegvaliase
- To characterize the long-term immunogenicity profile of pegvaliase in subjects who are administered pegvaliase using PFS drug presentation

Outcomes/endpoints

Primary endpoint in part 2:

- Change from Baseline in Blood Phenylalanine Concentration at Week 8.

Secondary endpoint in part 2:

- Secondary Efficacy Endpoint Analysis: Change in Neurocognitive and Neuropsychiatric Symptom Scores from Part 2 Baseline to Week 8.
- Change in ADHD-RS (investigator-rated inattention score) in subjects with BMN165-301 Baseline score >9 from Part 2 Baseline to Part 2 Week 8
- Change in ADHD-RS (investigator-rated inattention score) from Part 2 Baseline to Part 2 Week 8
- Change from Part 2 Baseline in PKU-specific POMS (self-rated) confusion subscale score from Part 2 Baseline to Part 2 Week 8

- Change from Part 2 Baseline in PKU-specific POMS (self-rated) TMD score from Part 2 Baseline to Part 2 Week 8
- Change from Part 2 Baseline in POMS (self-rated) from Part 2 Baseline to Part 2 Week 8

Tertiary Efficacy Endpoints in part 2:

- Dietary Intake
- Total Score and Hyperactivity/Impulsivity Subscale Score (ADHD-RS HI)
- POMS Subscale Scores

In part 1 of the study efficacy was not studied. This part was only used to select patients eligible for part 2, the randomised double-blind placebo-controlled withdrawal phase.

Part 3 pertained to PK.

Part 4 is the long term follow-up in which both phenylalanine and Neurocognitive and Neuropsychiatric Symptom Scores were collected.

Sample size

For Part 2, approximately 72 subjects (approximately 48 subjects for the active groups and approximately 24 subjects for the placebo groups) provided 97% power to detect a statistically significant difference in the primary endpoint between the pooled active group and the pooled placebo group at Week 8 of Part 2 with a Type I error rate of 0.05 (2-sided).

The following assumptions were made regarding blood Phe level by Week 8 of Part 2:

- By Week 8 of Part 2, subjects in the pooled active group maintained mean blood Phe concentrations ≤700 µmol/L with a common SD of 400 µmol/L
- By Week 8 of Part 2, subjects in the pooled placebo group increased mean blood Phe concentration to≥1100 µmol/L (after withdrawal from their pegvaliase dosing regimen) with a common SD of 400 µmol/L

A total of 72 subjects provided approximately 70% power for the ADHD RS-IV Inattention Subscale score to detect the estimated difference between the active groups and the placebo groups in Part 2 using the sequential procedure for multiplicity adjustment within the secondary endpoints. The following assumptions were made:

- There was a mean difference between the active group and the placebo group in the
- subset of subjects with a baseline ADHD RS-IV Inattention Subscale score >9
- (per baseline from 165-301) of 5 points with a SD of 5.5 points in the ADHD RS-IV
- (Investigator-Rated) Inattention Subscale score

And

- Fifty percent (50%) of the mITT Population had a baseline ADHD RS-IV Inattention
- Subscale score >9 (per baseline from the 165-301)

Randomisation

Upon entry into Part 1, subjects were administered open-label pegvaliase at a dose of either 20 mg/day or 40 mg/day, 7 days/week:

- Subjects who completed 165-301 continued with the same pegvaliase dose to which they were randomized in 165-301 (20 mg/day or 40 mg/day).
- Subjects who completed PAL-003 or 165-205 were randomized (1:1) to receive 20 mg/day or 40 mg/day pegvaliase (7 days per week). Subjects were randomized using an interactive web response system (IWRS).

Eligible subjects for Part 2 of the Study were randomized (2:1) in a double-blind manner upon entry into Part 2 using an IWRS to continue with their Part 1 daily pegvaliase dose (20 or 40 mg/day) or receive a daily matching placebo:

- Subjects who received 20 mg/day in Part 1 were randomized (2:1) to 20 mg/day pegvaliase (20 mg/day active group) or placebo (20 mg/day placebo group);
- Subjects who received 40 mg/day in Part 1 were randomized (2:1) to 40 mg/day pegvaliase (40 mg/day active group) or placebo (40 mg/day placebo group).

Randomization in Part 2 was stratified by mean blood phenylalanine measurement using the last two consecutive blood phenylalanine assessments in Part 1 (≤600 µmol/L or > 600 µmol/L) and ADHD RS-IV Inattention Subscale score, using the baseline from 165-301 (< 12 or > 12 or missing [or missing for subjects who initiated pegvaliase in a Phase 2 study]). The randomization schedule was developed by an independent third-party vendor (Section 4) to maintain the blinding of study drug assignment; BioMarin and site personnel were blinded to study drug assignment in Part 2.

Blinding (masking)

This was a double-blind study. Pegvaliase and placebo packaging were identical in appearance.

Statistical methods

Descriptive summaries of continuous variables included number of subjects (n), the mean, standard deviation (SD), median, minimum, maximum, and 95% confidence interval ([95% CI] as appropriate) for the mean. Descriptive summaries of categorical variables included number of subjects (n), frequency, and percent.

The baseline value for assessments in Part 2 was defined as the last available measurement prior to first administration of study drug in Part 2 unless otherwise specified.

Data from 165-301 were integrated with data in this study for the long-term safety and efficacy analyses.

Statistical tests were two-sided at the 0.05 significance level, and all CIs were two-sided 95% unless otherwise specified. For analysis of the primary efficacy endpoint in Part 2, the Hochberg procedure was used for multiplicity adjustment. Between the primary and secondary efficacy endpoints and within the secondary efficacy points in Part 2, the sequential hypothesis testing procedure was used for multiplicity adjustment.

To explore the treatment effect of the 2 doses of pegvaliase, change from Baseline in blood phenylalanine concentration was analysed using a mixed-effect model repeated measures (MMRM) model.

If the poolability test result between the 40 mg/day and 20 mg/day placebo groups for the primary analysis method was not significant (P > 0.1), the change from baseline in blood phenylalanine concentration at Week 8 of Part 2 was compared between the pooled active group versus the pooled placebo group using the MMRM method, with study drug group (pegvaliase, placebo), visit, and study drug-by-visit interaction as factors adjusting for baseline blood phenylalanine concentration.

If the poolability test result was significant (P ≤ 0.1) between the 40 mg/day and 20 mg/day placebo groups for the primary analysis method, the change from baseline in blood phenylalanine concentration at Week 8 of Part 2 was compared between the pooled active group versus the 20 mg/day placebo group and between the pooled active group versus the 40 mg/day placebo group using the MMRM method with study drug group (pegvaliase, placebo), visit, and study drug-by-visit interaction as factors adjusting for baseline blood phenylalanine.

An MMRM analysis was also performed for the population of subjects who entered the Maintenance Period and used at least 80% of their planned study medication during the Maintenance Period. Given the induction/titration/maintenance design of the study, an analysis population of subjects who reached their randomized dose level and demonstrated treatment compliance is better suited than the ITT population for assessing the actual treatment difference between the dose levels.

An ANCOVA model was also used to compare the treatment effect from Baseline to end of study for subjects in the 2 randomized dose groups who completed the Maintenance Period.

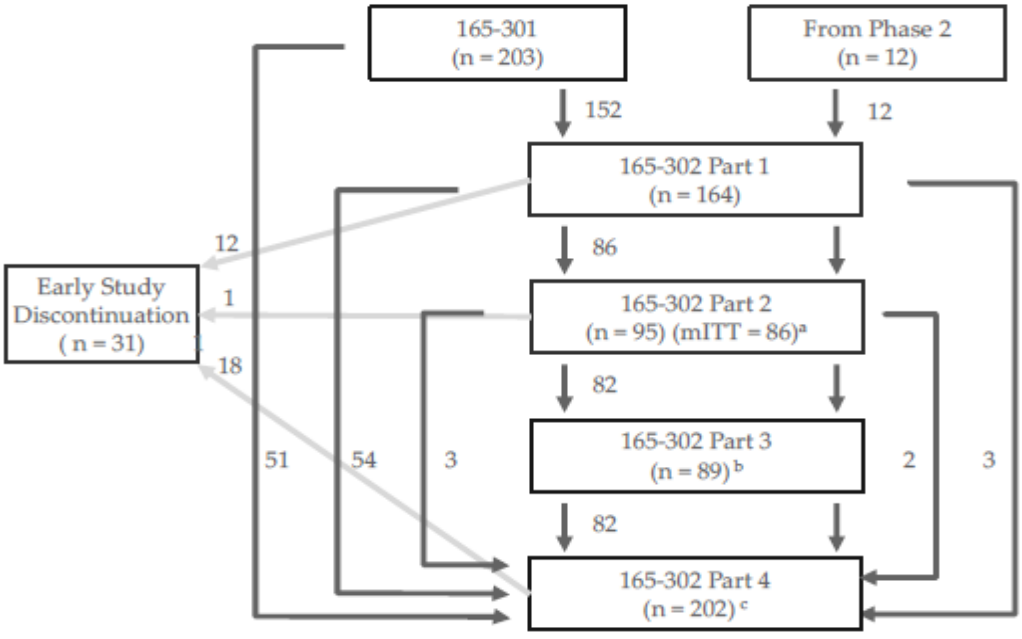
The primary analysis method for blood phenylalanine concentration was repeated in the mITT Population using the multiple imputation method and LOCF imputation as sensitivity analyses. Multiple imputation assumes that data are missing at random (MAR).

Results

Participant flow

An overview of the overall subject disposition is displayed in **Figure 14**.

Figure 14. Disposition of patients in Study 165-302



[a] *Subjects were not included in the primary efficacy analysis (mITT Population) if they did not have a mean blood Phe reduction of ≥ 20% (using the last two consecutive blood Phe assessments of Part 1) from baseline levels per Amendment #2. Some subjects had already enrolled into Part 2 when this criterion for inclusion in the mITT was implemented with Amendment #2 of the protocol.*

[b] *Only subjects who were enrolled under Amendment #2 were considered for the PK/PD analyses; 58 of the 89 subjects who entered Part 3 were enrolled under Amendment #2 and were included for the PK and PD analyses.*

[c] *A total of 51 subjects entered Part 4 directly from 165-301 because they did not achieve target dose in 165-301 (n = 33) or were affected by closure of enrolment into Part 2 (n = 18) after the target enrolment had been met.*
Another 57 subjects entered directly from Part 1 because they did not meet the blood Phe reduction criterion to qualify for entry into Part 2 (n = 39), due to closure of enrolment into Part 2 (n = 9), or for other reasons as instructed by the sponsor (n = 9). Five additional subjects entered Part 4 directly from Part 2 due to AEs.

Recruitment

Study initiation date: 29 July 2013 (first patient enrolled)

Study completion date: 23 September 2016 (last dose given)

Conduct of the study

There were three main amendments to the original protocol.

The protocol was amended (Amendment #1) on 10 January 2014. A total of 34 subjects enrolled into Part 1 of the study under Amendment #1. Changes with this amendment did not affect part 2 of the study.

The protocol was further amended (Amendment #2) on 8 August 2014; significant changes were made to the study design to better meet the study objectives and monitor subject safety. A total of 82 subjects enrolled into Part 1 and an additional 51 subjects enrolled directly in Part 4 of the study under amendment #2. The administrative changes introduced with this amendment were:

- Subjects from PAL-003 were only eligible for participation in the open-label extension (Part 4) of this study to receive pegvaliase at a dose of 10, 20, 40, or 60 mg/day, depending on the dose in which they completed PAL-003;

- the statistical analyses were modified to align with the changes made to the study design. The primary analysis was to be based on an mITT Population, which was defined as the combined analyses of all subjects randomized in Part 2 of the study with a mean blood phenylalanine reduction of ≥ 20% (using the last two consecutive blood phenylalanine assessments of Part 1) from baseline levels of 165-301.

An additional administrative letter was issued to sites (dated 28MAR2014) to temporarily suspend dosing of 60 mg/day pegvaliase following a request form the FDA.

The protocol was finally amended (Amendment #3) on 15 December 2015. Enrolment into the study had closed at the time of Amendment #3. The main changes with this amendment were :

o The sample size for Part 2 (RDT) of the study was adjusted due to limited enrolment opportunity of subjects from the adult PKU patient population in the United States.

o The primary and secondary analyses were revised to align with the change in sample size for Part 2 (RDT). Rather than performing separate comparisons for the 20 mg/day group and 40 mg/day group versus pooled placebo, pooling of subjects in the active groups (20 mg/day and 40 mg/day) versus pooled placebo was to be implemented.

Baseline data

Baseline characteristics and demographics prior to initiating dosing with pegvaliase are summarized in **Table 15** for the subjects included in the primary analysis (mITT Population).

Table 15. Demographics and Baseline Characteristics at part 2 (mITT Population, Study 15-302)

Baseline Characteristics at Part 2	**20mg/day Active**	**40mg/day Active**	**20mg/day Placebo**	**40mg/day Placebo**	**Pooled Active**	**Pooled Placebo**
Baseline	(N=29)	(N=29)	(N=14)	(N=14)	(N=58)	(N=28)
Weight, kg						
n	29	29	14	14	58	28
Mean (SD)	81.9 (19.40)	78.3 (21.87)	95.1 (26.99)	76.2 (16.18)	80.1 (20.57)	85.7 (23.86)
Median	80.2	75.3	88.4	71.6	79.8	81.1
Min , Max	49.4 , 130.4	42.4 , 134.2	50.4 , 143.6	58.1 , 111.4	42.4 , 134.2	50.4 , 143.6
Height, cm						
n	29	29	14	13	58	27
Mean (SD)	168.7 (6.70)	166.8 (9.90)	168.9 (9.96)	168.6 (9.31)	167.8 (8.43)	168.7 (9.47)
Median	169.0	163.8	165.8	168.1	167.8	166.0
Min , Max	153.0 , 179.3	149.9 , 186.2	154.9 , 184.5	155.0 , 181.3	149.9 , 186.2	154.9 , 184.5
BMI, kg/m2						
n	29	29	14	13	58	27
Mean (SD)	28.8 (6.65)	27.9 (6.63)	33.0 (7.53)	26.4 (4.45)	28.3 (6.59)	29.9 (6.98)
Median	28.0	26.5	33.2	25.6	27.5	28.9
Min , Max	19.4 , 48.2	17.3 , 41.6	20.5 , 44.4	19.9 , 33.9	17.3 , 48.2	19.9 , 44.4
<25	9 (31.0%)	12 (41.4%)	3 (21.4%)	5 (35.7%)	21 (36.2%)	8 (28.6%)
>=25 to <30	8 (27.6%)	7 (24.1%)	2 (14.3%)	5 (35.7%)	15 (25.9%)	7 (25.0%)
>=30	12 (41.4%)	10 (34.5%)	9 (64.3%)	3 (21.4%)	22 (37.9%)	12 (42.9%)
Missing	0	0	0	1 (7.1%)	0	1 (3.6%)
Blood phenylalanine Concentration, umol/L						
n	29	29	14	14	58	28
Mean (SD)	596.8 (582.75)	410.9 (439.95)	563.9 (504.62)	508.2 (363.68)	503.9 (520.28)	536.1 (432.54)
Median	666.0	216.0	660.5	584.5	259.0	638.0
Min , Max	0.0 , 1721.0	0.0 , 1247.0	0.0 , 1318.0	0.0 , 1021.0	0.0 , 1721.0	0.0 , 1318.0
ADHD-RS IA						
n	28	25	14	14	53	28
Mean (SD)	5.9 (4.70)	6.0 (6.45)	5.0 (4.26)	2.9 (3.68)	5.9 (5.54)	3.9 (4.05)
Median	5.5	3.0	4.0	1.5	4.0	2.0
Min , Max	0.0 , 13.0	0.0 , 19.0	0.0 , 12.0	0.0 , 11.0	0.0 , 19.0	0.0 , 12.0
ADHD-RS IA in Subjects with Naive baseline > 9						
n	15	11	5	6	26	11
Mean (SD)	7.7 (4.10)	7.3 (6.80)	8.0 (3.94)	4.7 (4.50)	7.5 (5.29)	6.2 (4.40)
Median	9.0	3.0	7.0	3.5	8.5	6.0
Min , Max	0.0 , 13.0	0.0 , 19.0	3.0 , 12.0	0.0 , 11.0	0.0 , 19.0	0.0 , 12.0
ADHD-RS HI						
n	23	21	11	9	44	20
Mean (SD)	6.1 (5.00)	5.7 (5.37)	2.8 (2.56)	3.0 (3.04)	5.9 (5.12)	2.9 (2.71)
Median	5.0	4.0	2.0	2.0	4.5	2.0

Min , Max	0.0 , 17.0	0.0 , 18.0	0.0 , 8.0	0.0 , 9.0	0.0 , 18.0	0.0 , 9.0
POMS TMD Observer-rated						
n	21	20	12	11	41	23
Mean (SD)	12.3 (27.02)	16.7 (22.52)	9.4 (21.19)	19.9 (23.40)	14.4 (24.71)	14.4 (22.41)
Median	11.0	13.5	5.5	19.0	12.0	6.0
Min , Max	-20.0 , 93.0	-14.0 , 76.0	-15.0 , 58.0	-6.0 , 55.0	-20.0 , 93.0	-15.0 , 58.0
POMS TMD Self-rated						
n	29	25	14	14	54	28
Mean (SD)	16.2 (35.63)	24.3 (35.38)	18.1 (28.26)	13.3 (21.63)	19.9 (35.41)	15.7 (24.81)
Median	2.0	12.0	22.5	12.5	8.0	15.0
Min , Max	-24.0 , 129.0	-15.0 , 107.0	-15.0 , 67.0	-19.0 , 65.0	-24.0 , 129.0	-19.0 , 67.0
PKU POMS TMD						
n	29	25	14	14	54	28
Mean (SD)	7.1 (14.18)	8.9 (12.28)	8.6 (10.84)	5.0 (8.56)	8.0 (13.24)	6.8 (9.76)
Median	2.0	6.0	10.5	5.0	4.0	7.5
Min , Max	-11.0 , 46.0	-6.0 , 33.0	-4.0 , 27.0	-7.0 , 25.0	-11.0 , 46.0	-7.0 , 27.0
PKU POMS Confusion Subscale						
n	29	25	14	14	54	28
Mean (SD)	2.0 (2.09)	2.4 (2.02)	2.1 (1.49)	1.2 (1.53)	2.2 (2.04)	1.6 (1.54)
Median	2.0	2.0	2.0	1.0	2.0	1.0
Min , Max	0.0 , 8.0	0.0 , 7.0	0.0 , 4.0	0.0 , 5.0	0.0 , 8.0	0.0 , 5.0
Protein from Intact Food						
n	29	29	14	14	58	28
Mean (SD)	50.6 (19.38)	47.4 (27.85)	38.1 (26.42)	39.4 (22.69)	49.0 (23.84)	38.7 (24.18)
Median	48.7	42.4	25.2	36.1	43.0	31.2
Min , Max	22.0 , 96.2	6.1 , 127.0	12.4 , 94.7	8.1 , 77.4	6.1 , 127.0	8.1 , 94.7
Protein from Medical Food						
n	29	29	14	14	58	28
Mean (SD)	8.9 (16.82)	17.4 (21.72)	20.7 (26.39)	28.4 (26.52)	13.2 (19.72)	24.5 (26.25)
Median	0.0	3.3	16.9	25.0	0.0	19.1
Min , Max	0.0 , 60.0	0.0 , 63.3	0.0 , 90.0	0.0 , 73.2	0.0 , 63.3	0.0 , 90.0
Protein Intake						
Restricted Diet (%)	0	1 (3.4%)	1 (7.1%)	3 (21.4%)	1 (1.7%)	4 (14.3%)

[a] *Under Amendment #1 (10JAN2014) of the study protocol, subjects ≥ 16 years old were eligible for study participation;*

[b] *One subject in the 40 mg/day placebo group did not have weight and height measured at baseline in 165-301;*

[c] *One subject had a naïve baseline blood phenylalanine level < 600 µmol/L, which was different from the blood phenylalanine level assessed at the time of screening for eligibility into this study;*

[d] *The ADHD RS-IV Hyperacitivity/Impulsitivity Subscale and POMS tools were not performed in 165-301 until the first protocol amendment; only subjects who had baseline assessments were included. Possible scores for the ADHD RS-IV Inattention and Hyperactivity/Impulsivity Subscales range from 0 to 27, with higher scores indicative of more severe symptoms. Possible scores for the POMS TMD range from -32 to 200, scores for the PKU POMS TMD range from -12 to 58, and scores for the PKU POMS Confusion Subscale range from 0 to 11, with higher scores indicative of more severe symptoms. Neurocognitive and neuropsychiatric tools were not administered in the Phase 2 studies;*

[e] *RDA for total protein for adults in the general population is 0.75 g/kg. For an 80-kg individual, approximately 60 g of daily protein is recommended;*

[f] *Subjects were considered to be on restricted protein intake if > 75% of total daily protein intake was from medical food. Total daily protein intake was the sum of daily protein intake from medical food and daily protein intake from intact food.*

Numbers analysed

The following study populations were defined:

Intent-to-Treat Population (Part 2): The ITT Population consisted of all subjects who were randomized into Part 2 of the study.

Modified Intent-to-Treat Population (Part 2): The mITT Population consisted of all subjects who reached the randomized dose of 20 mg/day or 40 mg/day and were randomized into Part 2 with a mean blood phenylalanine reduction of ≥ 20% (using the last two consecutive blood phenylalanine assessments of Part 1) from the baseline of 165-301 (or the Phase 2 study in which they initiated pegvaliase). Primary analyses of the primary and secondary endpoints were performed based on the mITT Population.

Per-Protocol Population (Part 2): The PP Population consisted of all subjects in the mITT Population who were compliant with the protocol with no major protocol violations that were considered to have affected efficacy analysis as detailed in the SAP. All efficacy analyses performed on the PP Population in Part 2 were based on the randomized study drug assignment.

RDT and Non-RDT Population (Part 4): The long-term analysis was performed for all subjects who were in the 165-301 ITT Population and who entered into Part 4 of this study regardless of participation in Part 2 of the study:

- The RDT Population was defined as all subjects in the mITT Population who were originally enrolled in 165-301, were randomized into Part 2, and entered Part 4.
- The Non-RDT Population was defined as all subjects who entered Part 4, were originally enrolled in 165-301, and did not participate in Part 2 because they did not have ≥20% blood phenylalanine reduction.
    - Subjects who enrolled directly into Part 4 of this study from 165-301 were included in the Non-RDT Population if they did not have ≥20% blood phenylalanine reduction (using the last two consecutive blood phenylalanine assessments) from the naïve baseline of 165-301.

Pharmacokinetic Population: The Part 3 PK Population consisted of all subjects with at least one PK measurement in Part 3 who were enrolled under Amendment #2. The Part 3 PK Population was analysed according to the study drug received (i.e. as treated).

Population for Safety Analyses: The population for safety analyses consisted of all subjects who enrolled into the study and all subjects who enrolled into each study part for analysis by study part. As part of the eligibility criteria for this study, subjects were to have completed a previous pegvaliase study (165-301, PAL-003, or 165-205) where they would have received pegvaliase as an induction and titration regimen (165-301 and 165-205) or as an adjustable dose regimen (PAL-003). All safety analyses were performed overall and separately for each part (Part 1, Part 2, Part 3 for PK/PD, and Part 4 for the long-term extension) of the study.
For Part 2, the safety population consisted of all subjects who were randomized and received study drug in Part 2; safety was analysed according to the study drug assignment actually received (i.e., as treated).

Outcomes and estimation

To assess primary and secondary efficacy endpoints, testing for the poolability of the placebo groups (20 mg/day and 40 mg/day) was performed and indicated a significant difference (P = 0.0424) at a pre-specified significance level of 0.1 between the 20 mg/day and 40 mg/day placebo doses for change from Part 2 baseline blood Phe concentration at Week 8 of Part 2 (data not shown).

Changes in blood phenylalanine concentration are presented for the pooled active group versus the 20 mg/day placebo group and the 40 mg/day placebo group (**Table 16**).

Table 16. Mixed-Model Repeated Measures of Change from Baseline in Blood Phenylalanine Concentration (µmol/L) at Week 8 of Part 2 (mITT Population, Study 165-302)

Part 2 Randomized Study Drug Group	n	Part 2 Baseline Mean (SD)	Part 2, Week 8 Mean (SD)	Mean (SD) Change from Part 2 Baseline	LS Mean Change from Part 2 Baseline (95% CI)	Difference in LS Means (95% CI)	P-value [a]
Pooled Active	58	503.9 (520.28)	559.2 (569.47)	18.6 (279.43)	26.50 (-68.26 to 121.26)		
20 mg/day Placebo	14	563.9 (504.62)	1509.0 (372.64)	996.4 (555.00)	949.75 (760.38 to 1139.11)	-923.25 (-1135.04 to -711.46)	<0.0001
Pooled Active	58	503.9 (520.28)	559.2 (569.47)	18.6 (279.43)	26.50 (-68.26 to 121.26)		
40 mg/day Placebo	14	508.2 (363.68)	1164.4 (343.32)	599.0 (507.40)	664.77 (465.45 to 864.10)	-638.27 (-858.97 to -417.57)	<0.0001

CI, confidence interval; LS, least squares (mean); mITT, modified Intent-to-Treat (Population); SD, standard deviation.
The mITT Population consisted of all subjects who reached the randomized dose of 20 mg/day or 40 mg/day and were randomized into Part 2 with a mean blood phenylalanine reduction of ≥ 20% (using the last two consecutive blood phenylalanine assessments of Part 1) from the baseline of 165-301 (or the Phase 2 study in which they initiated pegvaliase).
Baseline blood phenylalanine concentration was defined as the last blood phenylalanine measurement collected in 165-302 on or prior to dosing on Day 1 of Part 2.
All Part 2 Week 8 (Day 56) assessments related to the primary endpoint were performed on or within one week prior to the target day, otherwise it was considered as a missing value for the analyses of the primary endpoint and the appropriated missing value imputation method(s) were applied.
[a] *Based on the mixed model repeated measures (MMRM) method, with study drug (pegvaliase, placebo), visit, and study drug-by-visit interaction as factors adjusting for baseline blood phenylalanine concentration.*

The individual dose level comparisons (20 mg/day active vs.placebo and 40 mg/day active vs. placebo) were also performed as sensitivity analyses (**Table 17**).

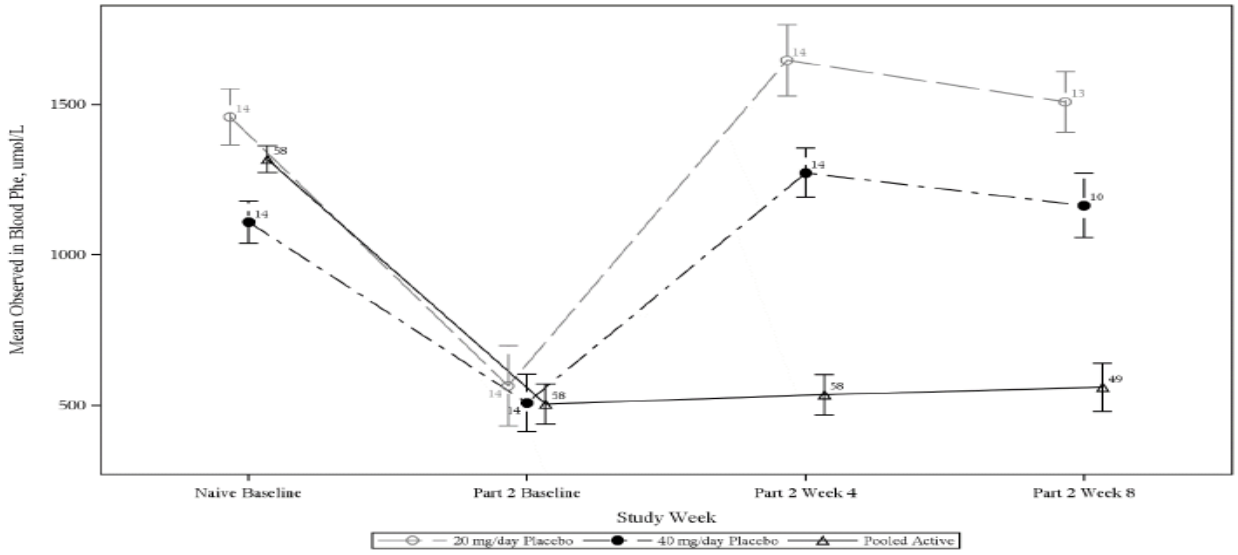
Table 17. Mixed-Model Repeated Measures of Change from Baseline in Blood Phe Concentration (µmol/l) at Week 8 of Part 2 for Poolability (mITT Population, Study 165-302)

Randomised study arm	Blood phenylalanine concentration (µmol/l)) Mean (SD)			LS mean change from Study 302 RDT baseline to Week 8 (95% CI)	Treatment difference in LS mean change (95% CI) P-value[2]
	Pre-treatment baseline[1]	Study 302 RDT baseline	Study 302 RDT Week 8		
Palynziq 20 mg once daily[3]	1450.2 (310.5) n = 29	596.8 (582.8) n = 29	553.0 (582.4) n = 26	-23.3 (-156.2, 109.7)	-973.0 (-1204.2, -741.9) p < 0.0001
Placebo 20 mg once daily[4]	1459.1 (354.7) n = 14	563.9 (504.6) n = 14	1509.0 (372.6) n = 13	949.8 (760.4, 1139.1)	
Palynziq 40 mg once daily[3]	1185.8 (344.0) n = 29	410.9 (440.0) n = 29	566.3 (567.5) n = 23	76.3 (-60.2, 212.8)	-588.5 (-830.1, -346.9) p < 0.0001
Placebo 40 mg once daily[4]	1108.9 (266.8) n = 14	508.2 (363.7) n = 14	1164.4 (343.3) n = 10	664.8 (465.5, 864.1)	

1 Blood phenylalanine level prior to initiating treatment with Palynziq
2 Based on the mixed model repeated measures (MMRM) method, with treatment arm, visit, and treatment arm-by-visit interaction (the time profile of blood phenylalanine changes is assessed separately for each treatment arm) as factors adjusting for baseline blood phenylalanine concentration.
3 Nine patients were excluded from the Week 8 analysis from the Palynziq treatment arms (20 mg/day or 40 mg/day): 4 patients did not complete the RDT due to adverse events (1 patient discontinued treatment and 3 patients transitioned to the long-term extension period) and the remaining 5 patients who did not complete phenylalanine assessment within the window for Week 8 (Day 43 to 56).
4 Five patients were excluded from the Week 8 analysis from the placebo arms (20 mg/day or 40 mg/day): 1 patient did not complete the RDT due to adverse event transitioned to the long-term extension period and the remaining 4 patients who did not complete phenylalanine assessment within the window for Week 8 (Day 43 to 56).

The change in observed blood Phe for the mITT Population was plotted from naïve baseline through open-label dosing in 165-301 or a Phase 2 study and Part 2 of this study. **Figure 15** shows the difference between the Part 2 dose groups, as well as the blood Phe concentrations for the placebo groups returning towards their naïve baseline blood Phe levels.

Figure 15. Mean (SE) Plot of Observed Blood Phe Concentration from Naïve Baseline through Part 2 (mITT Population)



Change in Neurocognitive and Neuropsychiatric Symptom Scores from Part 2 Baseline to Week 8 of Part 2

The secondary objective of Part 2 of this study was to evaluate the change from baseline in neurocognitive and neuropsychiatric symptoms of inattention and mood during Part 2, as measured by the ADHD RS-IV Inattention Subscale and the POMS tools (**Table 18**) in subjects previously exposed to pegvaliase who self-administer pegvaliase (20 or 40 mg/day) compared with those who self-administer matching placebo.

Table 18. Mixed-Model Repeated Measures of Change from Baseline in Neurocognitive and Neuropsychiatric Symptom Scores at Week 8 of Part 2 (mITT Population, Study 165-302)

Neurocognitive/Neuropsychiatric Symptom Scale	n [a]	Part 2 Baseline Mean (SD)	Part 2, Week 8 Mean (SD)	Mean (SD) Change from Part 2 Baseline	Change from Part 2 Baseline (95% CI)	Difference in LS Means 95% CI) [b]	P-value
ADHD RS-IV Inattention Subscale score (subjects with a 165-301 baseline score of >9), Part 2							
Pooled Active	26	7.5 (5.29)	9.9 (4.97)	2.5 (4.69)	3.05 (1.10, 5.00)	4.67 (-0.19 to 9.53)	0.0591
20 mg/day Placebo	5	8.0 (3.94)	7.8 (3.69)	-0.5 (2.52)	-1.62 (-6.07 to 2.83)		
Pooled Active	26	7.5 (5.29)	9.9 (4.97)	2.5 (4.69)	3.05 (1.10, 5.00)	2.77 (-1.99 to 7.52)	0.2447
40 mg/day Placebo	6	4.7 (4.50)	5.5 (2.65)	-1.0 (3.56)	0.28 (-4.05 to 4.61)		
ADHD RS-IV Inattention Subscale score, Part 2							
Pooled Active	58	5.9 (5.54)	6.8 (5.98)	0.8 (4.62)	1.24 (0.03, 2.45)	0.50 (-2.07, 3.06)	0.7007
20 mg/day Placebo	14	5.0 (4.26)	6.0 (4.58)	1.2 (3.00)	0.74 (-1.52, 3.01)		
Pooled Active	58	5.9 (5.54)	6.8 (5.98)	0.8 (4.62)	1.24 (0.03, 2.45)	1.64 (-1.16, 4.45)	0.2469
40 mg/day Placebo	14	2.9 (3.68)	3.2 (2.86)	-0.4 (3.44)	-0.40 (-2.93, 2.12)		
PKU POMS Confusion Subscale score, Part 2							
Pooled Active	58	2.2 (2.04)	2.4 (2.46)	0.3 (2.46)	0.59 (-0.08, 1.27)	-0.82 (-2.28, 0.63)	0.2612
20 mg/day Placebo	14	2.1 (1.49)	3.3 (2.72)	1.4 (2.47)	1.42 (0.14, 2.70)		
Pooled Active	58	2.2 (2.04)	2.4 (2.46)	0.3 (2.46)	0.59 (-0.08, 1.27)	-0.00 (-1.57, 1.56)	0.9969
40 mg/day Placebo	14	1.2 (1.53)	2.0 (2.00)	0.5 (2.22)	0.60 (-0.81, 2.01)		
PKU POMS TMD, Part 2							
Pooled Active	58	8.0 (13.24)	9.1 (14.43)	1.8 (12.01)	2.07 (-1.28, 5.42)	-3.09 (-10.31, 4.13)	0.3968
20 mg/day Placebo	14	8.6 (10.84)	12.4 (11.32)	4.3 (11.96)	5.16 (-1.24, 11.56)		
Pooled Active	58	8.0 (13.24)	9.1 (14.43)	1.8 (12.01)	2.07 (-1.28, 5.42)	0.08 (-7.59, 7.75)	0.9844

Neurocognitive/Neuropsychiatric Symptom Scale	n [a]	Part 2 Baseline Mean (SD)	Part 2, Week 8 Mean (SD)	Mean (SD) Change from Part 2 Baseline	Change from Part 2 Baseline (95% CI)	Difference in LS Means 95% CI) [b]	P-value
40 mg/day Placebo	14	5.0 (8.56)	6.8 (12.97)	0.0 (14.08)	2.00 (-4.90, 8.89)		
POMS TMD (Self-Rated), Part 2							
Pooled Active	58	21.6 (33.89)	22.9 (31.80)	4.0 (28.44)	4.15 (-3.06, 11.37)	-3.05 (-18.55, 12.45)	0.6963
20 mg/day Placebo	14	20.1 (26.58)	25.5 (26.21)	6.2 (25.77)	7.20 (-6.51, 20.92)		
Pooled Active	58	21.6 (33.89)	22.9 (31.80)	4.0 (28.44)	4.15 (-3.06, 11.37)	3.97 (-12.58, 20.52)	0.6342
40 mg/day Placebo	14	15.4 (20.12)	15.2 (23.63)	-1.1 (26.28)	0.19 (-14.70, 15.07)		

ADHD RS-IV, Attention Deficit Hyperactivity Disorder Rating Scale (Investigator-Rated); CI, confidence interval; ITT, Intent-to-Treat Population; LS, least squares (mean); mITT, Modified ITT Population; POMS, Profile of Mood States; TMD, Total Mood Disturbance (POMS).

Possible scores for the ADHD RS-IV Inattention and Hyperactivity/Impulsivity Subscales range from 0 to 27, with higher scores indicative of more severe symptoms.
Possible scores for the POMS TMD range from -32 to 200, scores for the PKU POMS TMD range from -12 to 58, and scores for the PKU POMS Confusion Subscale range from 0 to 11, with higher scores indicative of more severe symptoms.

All Part 2 Week 8 (Day 56) assessments related to the secondary endpoints were performed one or within one week prior to the target day, otherwise they were considered missing values for the analyses of the secondary endpoints and the appropriated missing value imputation method(s) were applied.
[a] Some subjects did not have Part 2 neurocognitive and neuropsychiatric assessments data collected (Section 8.2. The ADHD RS-IV Inattention Subscale, PKU POMS, and POMS tools were not performed in 165-301 until a protocol amendment; only subjects who had baseline assessments were included. Subjects who were included in the mITT Population from a Phase 2 study were not included because neurocognitive and neuropsychiatric tools were not administered in the Phase 2 studies.
[b] Negative values indicate a decline in symptom score (towards improvement); positive values indicate an increase in symptom score (towards decline).

Protein intake

Subjects were required to maintain a consistent level of protein intake during the study to ensure that changes in blood phenylalanine concentrations were attributable to the study drug rather than to changes in protein intake. Data were collected on subject-reported 3-day diet diaries and were assessed for its role in change in blood phenylalanine concentration relative to pegvaliase dosing during Part 2.

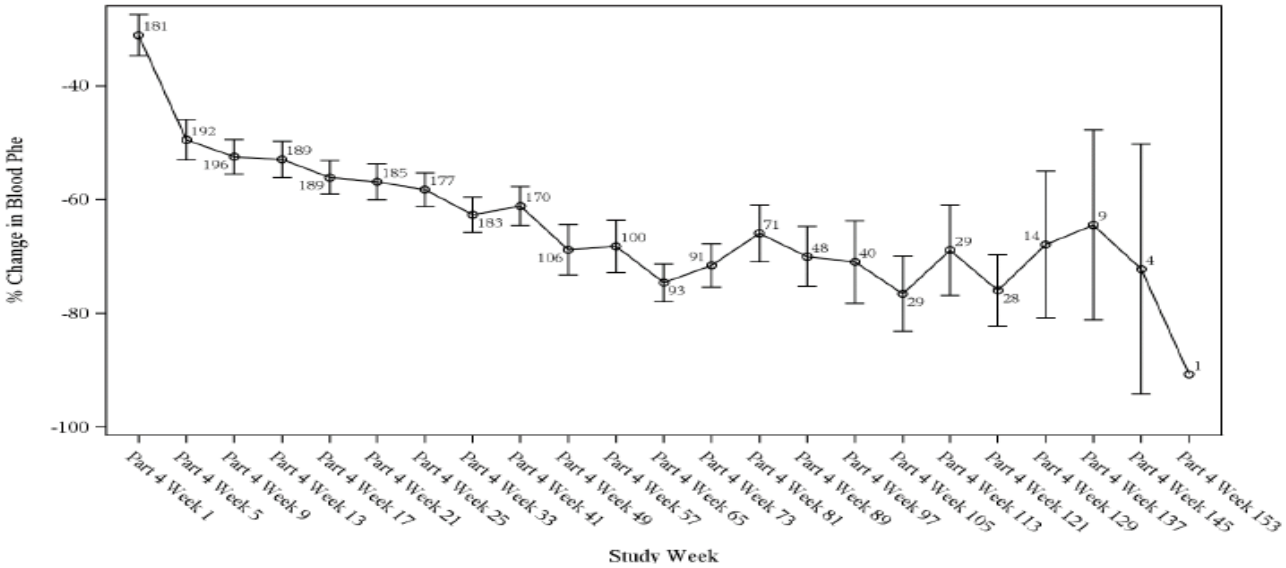
At the start of Part 2, daily protein intake from intact food for subjects randomized to the pooled active group was a mean (SD) 49.0 g (23.84) and a median of 43.0 g versus respective 38.1 g (26.42) and 25.2 g for subjects in the 20 mg/day placebo group and 39.4 g (22.69) and 36.1 g for subjects in the 40 mg/day placebo group. Subjects in the pooled active group maintained their mean (SD) daily protein intake from intact food at Week 8 with a mean 3.9% increase from Part 2 baseline (mean total intake of 47.1 g [24.06], median of 45.3 g).

Long-term efficacy

Analysis of the long-term efficacy of pegvaliase included experience from Part 4 of this study (the long-term, open-label extension phase).

Blood Phe concentrations are summarized for all doses administered in Part 4 and displayed in **Figure 16**.

Figure 16. Mean Plot of Change from Baseline in Blood Phenylalanine Concentration During Part 4 (All Subjects, Study 165-302)



The variability in sample size is attributed to the variability of subjects with available data at each time point (subjects who did not perform an assessment within the protocol-defined visit window, terminated from the study early, or have missing data were not included as of the cut-off for this Clinical Study Report). Subjects who did not have Week 1 data but had data for subsequent Part 4 time points were included. Part 4 results are based on data as of the data cut off for this CSR and are primarily discussed for subjects with data up through Week 41 of Part 4. These data and data at subsequent time points reflect a smaller and variable sample size due to the limited number of subjects with data at time points after Week 41 as of the data cut-off.

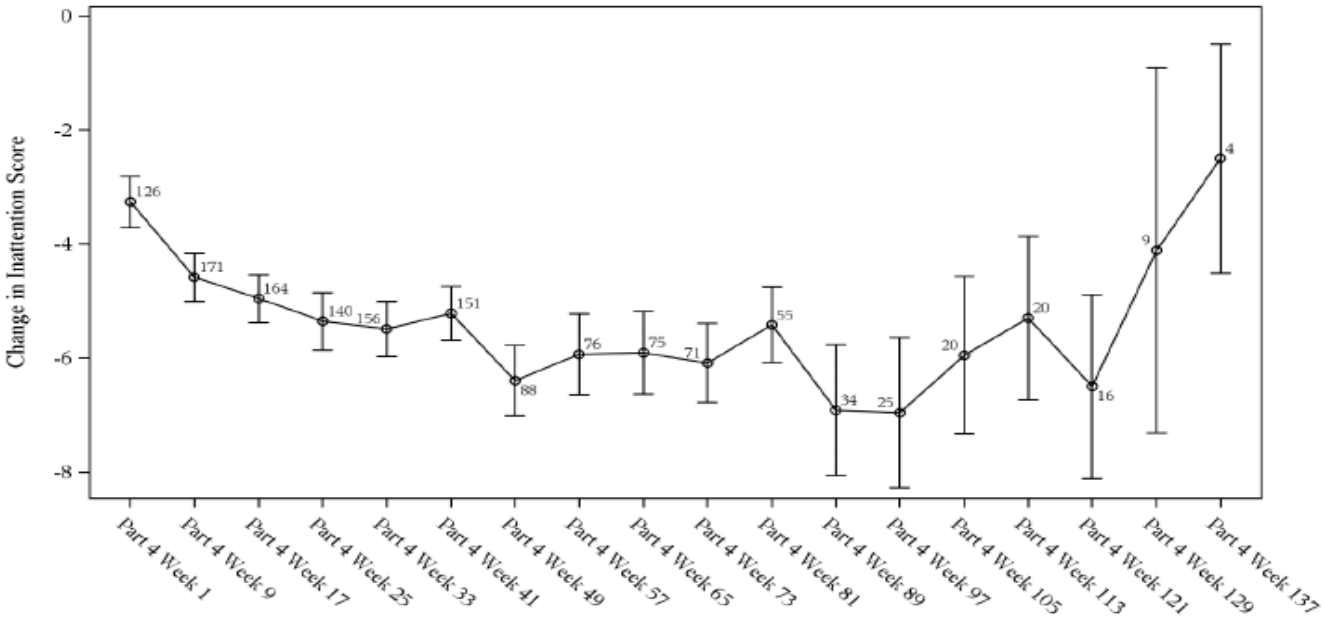
After 41 weeks of long-term, open-label pegvaliase dosing in Part 4 where adjustable dosing in response to blood Phe or tolerability was allowed , the majority of subjects who were able to continue long-term dosing to this time point were able to achieve blood Phe milestones at various doses, as summarized in **Table 19**.

Table 19. Percentage of Subjects Who Met Blood Phenylalanine Milestones at Week 41 of Part 4 (All Subjects)

Blood Phenylalanine Concentration Milestone at Week 41 of Part 4	**< 20 mg/day**	**20 to < 40 mg/day**	**40 to < 60 mg/day**	**≥ 60 mg/day a**	**Any Dose**
Percentage of subjects (n) with ≥ 20% blood phenylalanine reduction using last two blood Phenylalanine measures	88.2% (15/17)	88.0% (22/25)	74.7% (71/95)	66.7% (22/33)	76.5% (130/170)
Percentage of subjects (n) with blood phenylalanine reduction ≤ 600 µmol/L	88.2% (15/17)	84.0% (21/25)	64.2% (61/95)	42.4% (14/33)	65.3% (111/170)
Percentage of subjects (n) with blood phenylalanine reduction ≤ 360 µmol/L	82.4% (14/17)	76.0% (19/25)	54.7% (52/95)	36.4% (12/33)	57.1% (97/170)
Percentage of subjects (n) with blood phenylalanine reduction ≤ 120 µmol/L	76.5% (13/17)	56.0% (14/25)	40.0% (38/95)	21.2% (7/33)	42.4% (72/170)

Figure 17 show changes in the ADHD RS-IV Inattention Subscale scores over the duration of Part 4 for all subjects with observed data.

Figure 17. Mean Plot of ADHD RS-IV Inattention Subscale Scores and Change from Baseline During Part 4 (All Subjects)



Prior to initiating pegvaliase, mean (SD) protein intake from intact food for all enrolled subjects was 38.4 g (27.96 [n = 196]). Some increases were observed over the duration of Part 4 partially due to some subjects reaching low blood phenylalanine levels and increasing protein intake (within the protocol instructions).

Ancillary analyses

Additional analyses in support of prefilled syringe drug product presentation

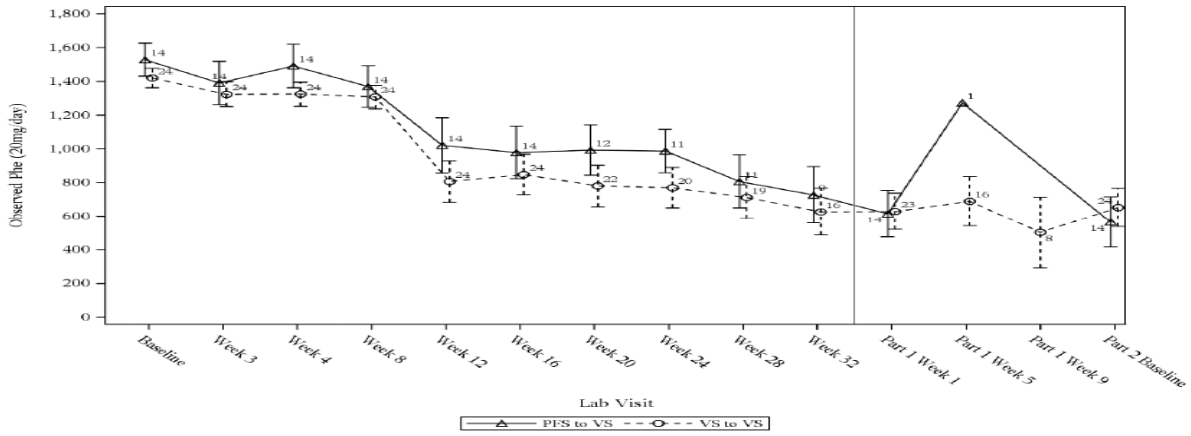
Results from PFS and VS comparability analyses performed in Part 3 of this study indicated that the exposure from pegvaliase per nominal dose delivered by PFS was 52% to 75% higher compared with pegvaliase delivered by VS based on the primary endpoints of $AUC_{0\text{-}4hr,ss}$ and $C_{max,ss}$. Because of this exposure difference observed between the two drug presentations, additional analyses were performed to assess the blood phenylalanine concentration, PK, PD, immunogenicity, and safety of subjects by PFS drug presentation (the intended to-be-marketed presentation) versus VS drug presentation. These additional analyses also included assessment of subjects who switched from PFS to VS pegvaliase at the start of 165-302 versus subjects who were exclusively administered VS into 165-302. Analyses were performed by 165-301 randomized dose group (20 and 40 mg/day) as well as all doses combined.

As subjects transitioned from 165-301 to 165-302, all subjects who entered Part 1 of 165-302 were to be administered pegvaliase as VS regardless of the drug presentation administered in 165-301.

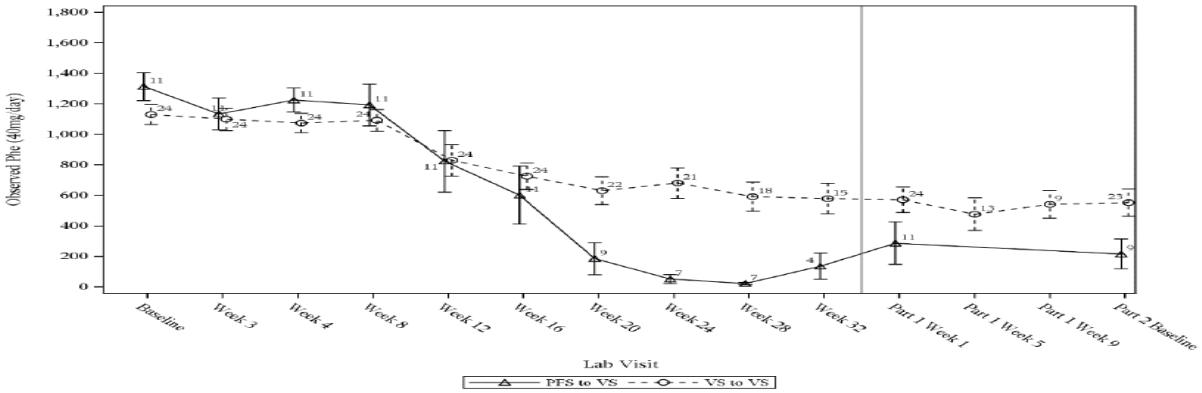
Blood phenylalanine concentration and trough pegvaliase levels were assessed for subjects in the primary analysis population (ie, the mITT Population who initiated pegvaliase and received exclusively either PFS or VS in 165-301) by the 20 and 40 mg/day dose groups and pegvaliase drug presentations (PFS to VS and VS only) to evaluate whether blood phenylalanine levels and trough pegvaliase concentrations were stable upon entry into the randomized discontinuation portion of 165-302 (Part 2). The observed blood phenylalanine levels from naïve baseline in 165-301 through Part 1 of this study are also displayed by 20 and 40 mg/day dose in **Figure 18**.

Figure 18. Mean (SE) Blood Phenylalanine Concentration over Time, Subjects Who Switched From Prefilled Syringe to Vial and Syringe Versus Subjects Who Remained on Vial and Syringe by Dose (mITT Population, 165-301 Subjects)

Panel A: 20 mg/day



Panel B: 40 mg/day



SE, standard error (of the mean)
Analyses were performed for subjects who exclusively used one or the other drug presentation in 165-301. Part 2 Visits to the left of the vertical reference line are those that occurred in 165-301; Visits to the right of the vertical reference line are those that occurred in 165-302.

Another population was defined to include the overall parent study 301 population (n=261, patients from parent study 301 who continued to 302), as psychological symptom efficacy endpoints (ADHD-RS IV, PKU-POMS and POMS scores), data on MNT status, and some immunogenicity data were collected for these patients but not for other subjects other subjects who started pegvaliase in a Phase 2 parent study (PAL-002, PAL-004, 165-205).

188 out the 261 patients received treatment for at least 1 year, 4 patients completed treatment, and 69 discontinued treatment in the first year. Of these 188 patients, 164 patients received treatment for at least 2 years, 2 patients continued treatment but had not yet reached 2 years of treatment, and 22 patients discontinued in the second year, and 9 patients discontinued after 2 years of treatment. Of the 100 patients who discontinued treatment, 40 patients discontinued due to an adverse event, 29 patients discontinued due to patient decision, 10 patients discontinued due to physician decision, and 21 patients discontinued to other reasons (e.g. lost to follow-up, pregnancy, or protocol deviation).

Efficacy results in this population are presented in **Table 20**.

Table 20. Efficacy results at Month 12, Month 18, Month 24 and Month 36 in pegvaliase-treated patients

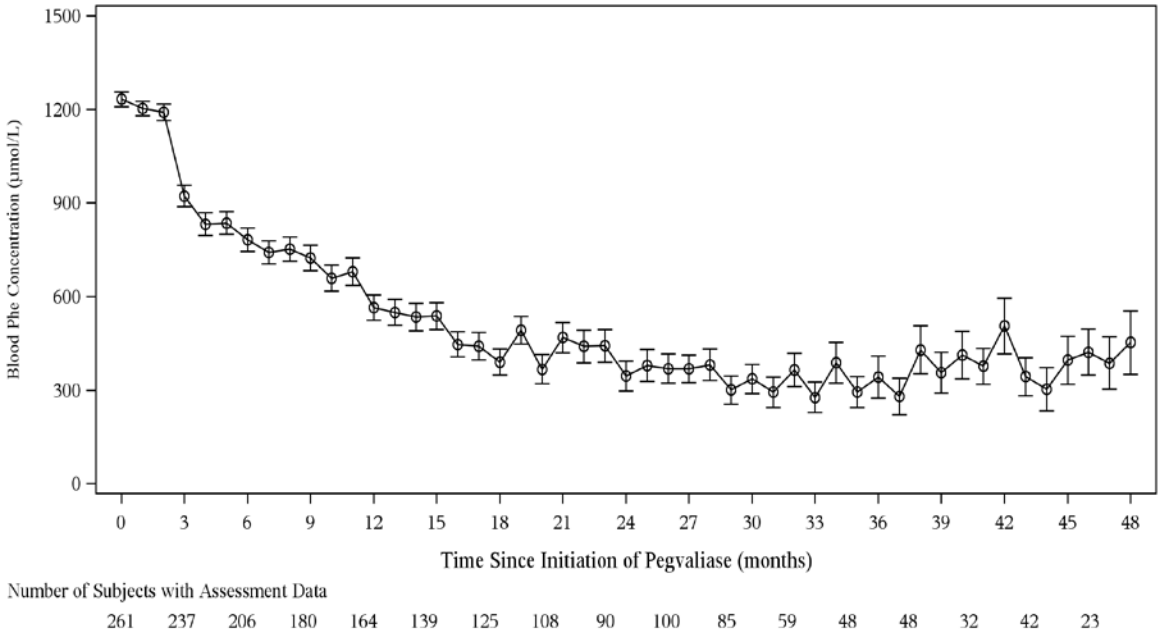
	Baseline	Month 12	Month 18	Month 24	Month 36
Blood phenylalanine[1]					
N	261	164[2]	125[2]	90[2]	48[2]
Mean (SD) blood phenylalanine (µmol/l)	1233 (386)	565 (531)	390 (469)	345 (453)	341 (465)
Change from baseline (µmol/l)l) Mean (SD) Median	-	 -662 (588) -634	 -883 (565) -920	 -873 (566) -965	 -956 (536) -913
ADHD inattention[3] subscale (investigator-rated)					
N	253	178	175	167	97
Mean (SD) inattention score	9.8 (6.1)	5 (4.9)	4.6 (4.7)	4.2 (4.6)	3.7 (5)
Change from baseline inattention score (n)[4] Mean (SD) Median	-	n=172 -4.7 (5.6) -4	n=168 -5.3 (5.9) -5	n=160 -5.9 (6.1) -5	n=92 -6.7 (6.4) -5.5
ADHD inattention[3] subscale (investigator-rated) with baseline score > 9					
N	116	80	78	76	45
Mean (SD) inattention score	15.3 (4.1)	7.6 (4.9)	6.6 (5)	5.9 (4.9)	5.1 (5.6)
Change from baseline inattention score (n)[4] Mean (SD) Median	-	n=80 -7.8 (5.5) -7	n=78 -8.9 (5.8) -9	n=76 -9.6 (5.9) -10	n=45 -10.6 (6.4) -12
PKU-POMS confusion[3] subscale (self-rated)					
N	170	181	178	169	100
Mean (SD) confusion score	4 (2.7)	2.4 (2.1)	2.1 (2.2)	2 (2.1)	1.8 (2.1)
Change from baseline confusion score (n)[4] Mean (SD) Median	-	n=130 -1.6 (2.5) -1	n=123 -2 (2.8) -2	n=117 -2.2 (2.7) -2	n=51 -2.2 (3.1) -2
Protein intake from intact food (g)					
N	250	160	111	84	46
Mean (SD)	39 (28)	47 (29)	50 (27)	54 (27)	72 (27)
Change from baseline protein intake (n)[4] Mean (SD) Median	-	n=154 9 (25) 4	n=106 12 (25) 9	n=81 16 (28) 14	n=44 27 (34) 25

[1] *Post-baseline phenylalanine values were mapped to the closest monthly visit (i.e. within a 1-month window).*
[2] *Reflects number of patients who reached time point (Month 12/Month 18/Month 24/Month 36) of treatment at the time of the data cut-off and had a scheduled phenylalanine assessment for that time point.*
[3] *Post-baseline ADHD-inattention/PKU-POMS confusion values were mapped to the closest 3-month visit (i.e. within a 3-month window).*
[4] *Change from baseline was based on subjects with available measurements at both time points. Not all subjects had a baseline ADHD inattention score and POMS confusion score taken at the start of the study.*

Phenylalanine levels over time

Mean blood phenylalanine levels reduced from 1233 µmol/l at baseline to 565 µmol/l at Month 12 (N=164) and 345 µmol/l at Month 24 (N=90), and these reductions in mean blood phenylalanine levels were maintained through Month 36 (341 µmol/l; N=48). Median change from baseline was -634 µmol/l at Month 12, -965 µmol/l at Month 24, and -913 µmol/l at Month 36 (**Figure 19**).

Figure 19. Mean Blood Phenylalanine Concentration Over Time (Subjects With Parent Study 165-301 Only_

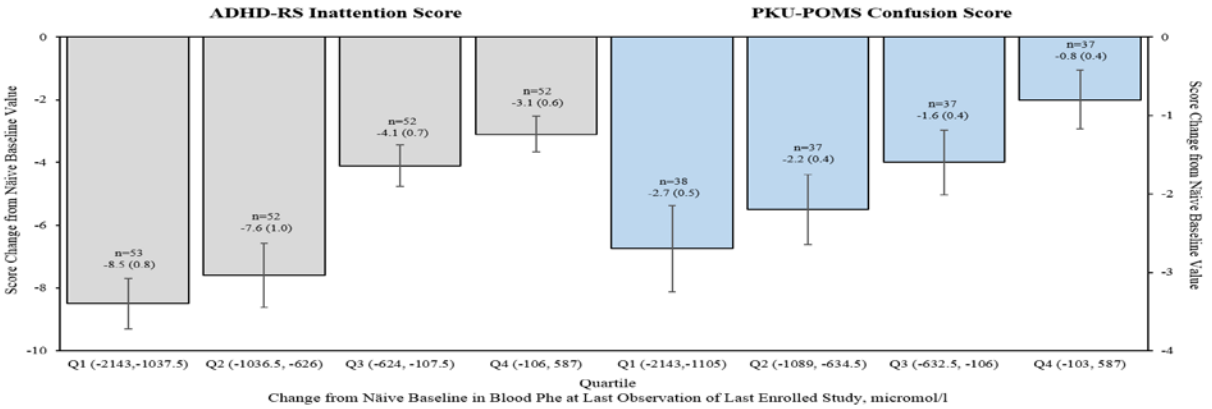


Changes in protein intake from intact food over time

Median protein intake from intact food increased at Month 12 (4 g increase from baseline), and Month 24 (14 g increase from baseline) and Month 36 (25 g increase from baseline).

An analysis of ADHD inattention and PKU-POMS confusion subscales by change in blood phenylalanine from baseline quartiles showed that patients with the largest phenylalanine reductions (Quartile 1) experienced the greatest improvements in ADHD inattention and PKU-POMS confusion subscales (see **Figure 20).**

Figure 20. Mean (SE) change from baseline in ADHD inattention and PKU-POMS confusion by change from baseline in blood phenylalanine quartiles at last observation of last enrolled study



As none of the clinical studies in the dossier were designed to assess pegvaliase response based on previous response to prior treatment, the applicant performed post-hoc analysis to support the claimed indication "*Palynziq is indicated for the treatment of adults with phenylketonuria (PKU) who have inadequate blood phenylalanine control (blood phenylalanine levels greater than 600 µmol/l) despite prior*

management with available treatment options ". Different patient populations were defined by the applicant. The data from the Principal MAA 3rd Line Subpopulation was compared with the data from the I/T/M Population.

The applicant compared the data of blood phenylalanine in the different populations: I/T/M Population (n=285), Principal MAA 3rd Line Subpopulation (n=180), and subpopulations: sapropterin non-responders (n=144), patients on a diet (>75% of daily food intake comes from MNT) (n=41) and sapropterin non-responders on MNT (n=57). The baseline demographics were balanced. Baseline phenylalanine values were balanced, except for those patients that are on >75% MNT. The subpopulation "s*apropterin non-responders on MNT*" reflects the proposed indication.

Baseline phenylalanine levels were 1331.2 (358.6) mean (SD) µmol/L in the Principal MAA 3rd Line Subpopulation, 1227.3 (379.3) µmol/L for the I/T/M Population, 901.2 (266.2) µmol/L in the sapropterin non-responders, 1116.3 (336.5) in patients on MNT with >75% from medical food and 1227.3 (379.3) µmol/L sapropterin non-responders on MNT.

In **Table 21** the change from baseline after 12 months of pegvaliase treatment in the different population is presented.

Table 21. Change from baseline phenylalanine in the different populations following 12 months of treatment

	MAA 3rd Line (n=180)	**I/T/M (n=285)**	**Sapropterin Non-Responders (N = 144)**	**Patients on MNT with >75% of protein intake from medical food (N = 41)**	**Sapropterin Non-responders on MNT (N = 57)**
n	111	184	93	29	36
Observed blood phenylalanine (µmol/L)					
Mean (SD)	525.5 (546.4)	546.6 (520.8)	502.5 (530.7)	494.1 (482.1)	475.4 (489.9)
≥20% Blood phenylalanine reduction from baseline, n (%)	86 (77.5%)	130 (70.7%)	73 (78.5%)	16 (55.2%)	27 (75.0%)
Blood phenylalanine reduction to ≤600 µmol/L, n (%)	67 (60.4%)	103 (56.0%)	58 (62.4%)	18 (62.1%)	23 (63.9%)
Change in blood phenylalanine level from baseline (µmol/L)					
median	-788.0	-653.0	-775.0	-487.0	-628.0
Percent change from baseline					
median	-73.5%	-58.8%	-73.5%	-52.1%	-67.3%

Summary of main study

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 22. Summary of Efficacy for trial 165-302, part 2

Title: a four-part, phase III, randomized, double-blind, placebo-controlled, four-arm, discontinuation study to evaluate the efficacy and safety of subcutaneous injections of pegvaliase self administered by adults with phenylketonuria.	
Study identifier	165-302

Design	*The study consisted of 4 parts (induction/maintenance (part 1; 13 weeks) ; a double blind placebo-controlled randomized withdrawal part (part 2; 8 weeks), a PK part (part 3; 6 weeks) and long term follow-up (part 4; up to 212 weeks)*		
	part 2	Duration of randomized withdrawal phase:	8 weeks
Hypothesis	Exploratory: specify		
Treatments groups	mITT[2]		**part 2**
	20 mg/day		29
	40 mg/day		29
	placebo 20 mg/day		14
	placebo 40 mg/day		14
Endpoints and definitions	**Primary endpoint in part 2**	blood phenylalanine level	change from baseline part 2 to week 8 in blood phenylalanine levels.
	part 2: secondary endpoint	neurocognitive/neuropsychiatric symptom scores[1]	i) ADHD-RS Inattention score ii) POMS Self-Reported Total Mood Disturbance Score iii) PKU-POMS Self-Reported Total Mood Disturbance Score iv) PKU POMS Self-Reported Confusion Score
Database lock	5 February 2018		

PART 2

Results and Analysis

Analysis description	**Primary Analysis**				
Analysis population and time point description	modified Intent to treat				
Primary endpoint. Change from baseline part 2 to week 8 in blood Phenylalanine. mean (SD)	Treatment group		Pooled active	Placebo 20 mg/day	Placebo 40 mg/day
	Number of patients		58	14	14
	mean		+18.6 µmol/L	+996.8 µmol/L	+599.0 µmol/L
	SD		279.4	555.0	507.4
Secondary endpoint. Change from baseline part 2 to week 8 in Neurocognitive and Neuropsychiatric Symptom Scores.	ADHD-RS-IV Inattention Subscale score	mean	0.8	1.2	-0.4
		SD	4.6	3.0	3.4

mean (SD)	PKU-POMS	mean	1.8	4.3	0
		SD	12.0	12.0	14.1
	PKU-POMS Confusion Subscale Scores	mean	0.3	1.4	0.5
		SD	2.5	2.5	2.2
	POMS Self-rated TMD Scores	mean	4.0	6.2	-1.1
		SD	28.4	25.8	26.3

Effect estimate per comparison	Primary endpoint: change from baseline part 2 in blood Phenylalanine	Comparison groups	pooled active - 20 mg/day placebo
		LS Mean Difference	-923.3
		95% CI	(-1135.04 to -711.46)
		P-value	<0.0001
		Comparison groups	pooled active - 40 mg/day placebo
		LS Mean Difference	-638.3
		95% CI	(-858.97 to -417.57)
		P-value	<0.0001
	Secondary endpoint: Change from baseline part 2 to week 8 in ADHD-RS-IV Inattention Subscale score	Comparison groups	pooled active - 20 mg/day placebo
		LS Mean Difference	0.50
		95% CI	(-2.07, 3.06)
		P-value	0.70
		Comparison groups	pooled active - 40 mg/day placebo
		LS Mean Difference	1.64
		95% CI	(-1.16, 4.45)
		P-value	0.25
	Secondary endpoint: Change from baseline part 2 to week 8 in PKU POMS Confusion Subscale score	Comparison groups	pooled active - 20 mg/day placebo
		LS Mean Difference	-0.82
		95% CI	(-2.28, 0.63)
		P-value	0.26
		Comparison groups	pooled active - 40 mg/day placebo
		LS Mean Difference	-0.00
		95% CI	(-1.57, 1.56)
		P-value	1.0
	Secondary endpoint: Change from baseline part 2 to week 8 in PKU POMS TMD	Comparison groups	pooled active - 20 mg/day placebo
		LS Mean Difference	-3.09
		95% CI	(-10.31, 4.13)
		P-value	0.40
		Comparison groups	pooled active - 40 mg/day placebo
		LS Mean Difference	0.08
		95% CI	(-7.59, 7.75)
		P-value	0.98
	Secondary endpoint:	Comparison groups	pooled active - 20 mg/day placebo

	Change from baseline part 2 to week 8 in POMS TMD (Self-Rated)	LS Mean Difference	-3.05
		95% CI	(-18.55, 12.45)
		P-value	0.70
		Comparison groups	pooled active - 40 mg/day placebo
		LS Mean Difference	3.97
		95% CI	(-12.58, 20.52)
		P-value	0.63
Notes	not applicable		
Analysis description	**tertiary analysis**		

	Treatment group	**active 20 mg/day**	**active 40 mg/day**	**Placebo 20 mg/day**	**Placebo 40 mg/day**
change from baseline to week 8 part 2: protein intake from intact food (grams)	median	0.7	-0.6	1.7	1.0
change from baseline to week 8 part 2: dietary phenylalanine intake	median	-5.7	-12.5	5.9	2.9

1) ADHD RS hyper was also analysed, however the ADHD-RS-IV inattention score is considered to be more important in adult patients (see clinical report).

2) mITT: patients who had a minimum blood phenylalanine efficacy of 20% reduction from naïve baseline (e.g., baseline study 165-301 or the Phase II study in which they initiated pegvaliase).

Table 23. Summary of efficacy for trial 165-302 (part 4).

Title: a four-part, phase III, randomized, double-blind, placebo-controlled, four-arm, discontinuation study to evaluate the efficacy and safety of subcutaneous injections of pegvaliase self administered by adults with phenylketonuria.					
Study identifier	165-302				
Design	*The study consisted of 4 parts (induction/maintenance (part 1; 13 weeks) ; a double blind placebo-controlled randomized withdrawal part (part 2; 8 weeks), a PK part (part 3; 6 weeks) and long term follow-up (part 4; up to 212 weeks)*				
	part 4	Duration of randomized withdrawal phase:	up to 212 weeks		
Hypothesis	Exploratory: open label				
Treatments groups	Treatment groups	**<20 mg/day**	**20-<40 mg/day**	**40-<60 mg/day**	**≥60 mg/day**
Endpoints and definitions	**secondary endpoint in part 4**	blood phenylalanine level	responder analysis %patients with blood phenylalanine ≤600 µmol/L.		
	secondary endpoint in part 4	blood phenylalanine level	change from baseline part 4 to week 41 in blood Phenylalanine levels.		
	tertiary endpoint in part 4	neurocognitive /neuropsychiatric symptoms	change from baseline study 165-301 to month 24		
Database lock	5 February 2018				
PART 4 (ongoing part of study 165-302)[5]					
Results and Analysis					
Analysis description	**Primary Analysis**				

Analysis population and time point description					
secondary endpoint: Change from baseline part 4 to week 41 (data cut off) in blood phenylalanine. mean (SD)	**Treatment group**	**<20 mg/day**	**20-<40 mg/day**	**40-<60 mg/day**	**≥60 mg/day**
	Number of patients	38	112	181	47
	mean	-1015.1	-1033.1	-733.7	-597.4
	SD	583.1	533.8	597.6	494.4
secondary endpoint: Percentage of patients (n) with 2 consecutive measures of blood phenylalanine ≤600 µmol/L	% (n)	88.2% (15/17)	84.0 (21/25)	64.2% (61/95)	42.4% (14/33)
Tertiary endpoint ADHD-RS inattention score Baseline part 4 to week 41	n	42	130	185	69
	median	-5.0	-3.0	-4.0	-5.0
Tertiary endpoint: ADHD-RS inattention score Baseline study 165-301 to month 24	n	19	23	84	34
	mean (SD)	-6.5 (6.7)	-6.0 (5.4)	-6.3 (6.0)	-4.2 (6.1)
Notes	Tertiary endpoints: For the PKU-POMS a similar trend is observed as for ADHD-RS. The mean (SD) change from baseline in ADHD-RS IA to months 12, 18 and 24 were -4.7 (5.6), -5.3 (5.9) and -5.9 (6.1) points, respectively. The mean (SD) change from baseline in ADHD-RS IA in subjects with baseline score of >9 at months 12, 18 and 24 were -7.8 (5.5), -8.9 (5.8) and -9.6 (5.9) points, respectively. The mean (SD) score change from baseline in the POMS self-rated TMD at months 12, 18 and 24 were -16.9 (32.6), -20.0 (34.2) and 20.1 (35.5) points, respectively The proportion of responders in the total population for ADHD-RS IA using this CIR threshold at 12, 18 and 24 months was 57/172 subjects (33.1%), 64/168 subjects (38.1%), and 67/160 subjects (41.9%). The proportion of responders in the ADHD-RS IA subscale in the subjects with a baseline score >9 at 12, 18 and 24 months was 42/80 subjects (52.5%), 50/78 subjects (64.1%), and 54/76 subjects (71.1%). • Mean (SD) protein intake from intact food • RDT patients: ranging from 42.1 (27.54) g at baseline to 71.2 (26.03) g at Week 105 • Non-RDT patients: ranging from 34.7 (25.82) g at baseline to 51.1 (30.26) g at Week 105.				

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

The applicant defined the Induction, Titration, Maintenance Population (N=285) which includes subjects whose overall pegvaliase treatment followed an induction, titration, and maintenance treatment regimen, similar to the proposed treatment regimen. This population includes data from subjects originally enrolled in parent studies 165-205 and 165-301 and data from the extension studies to which they transferred (PAL-003 and 165-302).

For the subjects in the I/T/M Population who reached and completed 12 months of treatment (184/285), blood Phe concentrations were reduced by a mean (SD) of 674.9 µmol/L (579.6; n = 184) to a mean (SD) blood Phe level of 546.6 (520.8) µmol/L at 12 months). Reductions to≤600 µmol/L (controlled Phe) were achieved by 56.0% (n = 103) and reductions to ≤ 120 μ mol/L (normalized Phe) were achieved by 37.0%

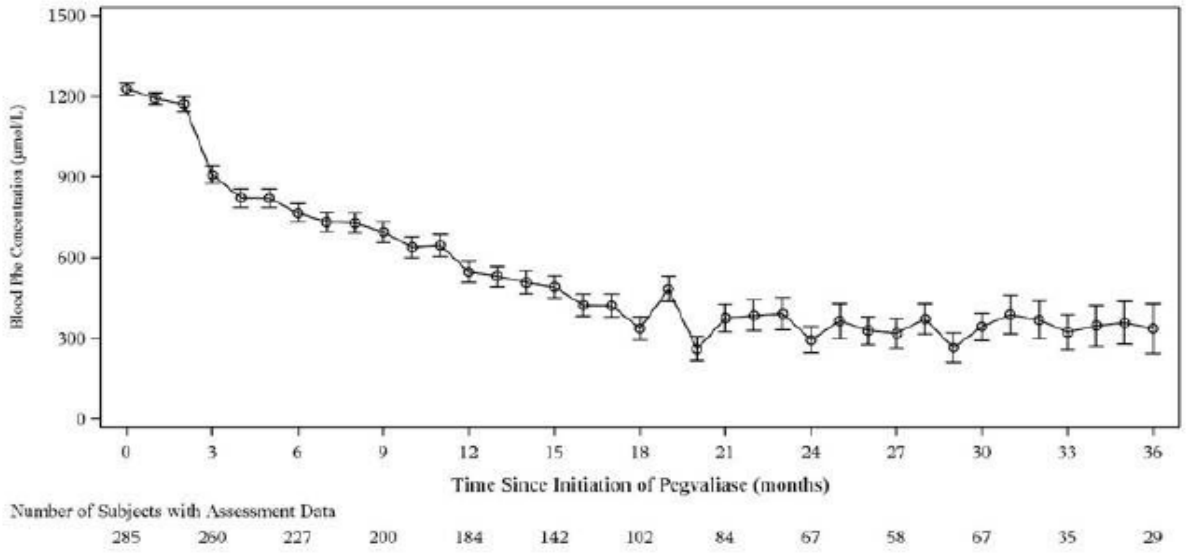
(n = 68).

For the subjects in the I/T/M Population who reached and completed 24 months of treatment (67/285), blood Phe concentrations were reduced by a mean (SD) of 913.6 µmol/L (528.3; n = 67) to a mean (SD) blood Phe level of 294.3 (398.0) µmol/L.

Reductions to ≤ 600 µmol/L (controlled Phe) were achieved by 82.1% (n = 55) and reductions to ≤ 120 μ mol/L (normalized Phe) were achieved by 50.7% (n = 34)

Mean (SE) Phe concentration over time is presented for the I/T/M Population in **Figure 21**.

Figure 21. Mean (SE) Plot of Blood Phe Concentration over Time Analysis Population: I/T/M (N=285)



Clinical studies in special populations

The applicant did not study pegvaliase in patients with hepatic and/or renal impairment.

No separate studies in patients over 65 years of age were submitted.

Supportive studies

PAL-003

Study PAL-003 is an ongoing long-term extension of a phase II, open-label, dose-finding study to evaluate the safety, efficacy, and tolerability of multiple subcutaneous doses of rAvPAL-PEG in patients with PKU. The study duration is up to 354 weeks.

Adults with PKU who had completed a previous Phase II study (PAL-002, PAL-004, 165-205) were eligible for participation in this study.

Eighty (80) patients from the preceding (parent) phase II studies (PAL-002 (n=40); PAL-004 (n=16) and 165-205 (n=24)) were enrolled. In total 68 patients were treated with pegvaliase.

Thirty-five (35) out of 80 (44%) of patients in the parent studies of PAL-003 (studies PAL-002 + PAL-004 + 165-205) had blood phenylalanine levels ≤600 µmol/L from baseline in the phase II studies. Under continued treatment in PAL-003 (either on the same dose or an increased dose to achieve the target phenylalanine level) an additional 24 patients showed phenylalanine levels ≤600 µmol/L (59/68 patients; 87%) from baseline phase II.

Up to week 192 (≈3.5 years) under continued pegvaliase treatment patients were capable to increase their natural protein intake whilst maintaining phenylalanine levels ≤600 µmol/L. After week 264 the patient numbers are too small to draw firm conclusions.

Study 165-303

Study 165-303 was a substudy of study 165-302. The study only included 9 patients (6 patients on active treatment and 3 patients on placebo in study 165-302 part 2). This study investigates neurocognitive and neuropsychiatric function of the patient based in the Cambridge Neuropsychological Test Automated Battery (CANTAB). However, given the limited number of patients included, no firm conclusions can be drawn from this study.

2.5.3. Discussion on clinical efficacy

Design and conduct of clinical studies

The clinical program for pegvaliase was conducted in US centres only. Given the fact that pegvaliase is an enzyme substitution therapy it is considered unlikely that differences would have been observed in an EU population.

The applicant defined different phases of treatment for the patients included in the clinical trials, namely induction, titration and maintenance (I/T/M).

During the induction phase of studies 165-205 and 165-301 (week 1 - 4) a fixed dose of 2.5 mg/week was used, in order to desensitise the patient to the risk of hypersensitivity reactions. This fixed dose was chosen as in the preceding phase II studies it was shown that when using a dose higher than 2.5 mg/week the clearance of pegvaliase was too high and no drug concentration could be measured.

Due to the doses and its duration, data collected in the induction phase of the various studies are important only from a safety perspective whereas, the maintenance phase data are considered pivotal to demonstrate efficacy of pegvaliase.

Study 165-301

The patients (n=261) enrolled in study 165-301 received a 4 week induction period and a titration varying from 5 to 30 weeks. Of those patients who reached the maintenance phase (n=195), 171 patients completed the maintenance phase at the assigned randomised dose (e.g. 20 or 40 mg/day). Twelve patients completed the randomised dose below or above the assigned dose. The sample size declined

from 261 at baseline to 80 at week 36. Efficacy assessments were collected up to 14 days from the date of the last dose taken. The reported effects are therefore on patients "while on treatment", rather than the more commonly accepted efficacy in an ITT population.

The study enrolled patients with phenylalanine levels >600 µmol/L. This included patients who had never received any treatment other than a restricted diet, patients who received sapropterin but still had phenylalanine levels ≤600 µmol/L), or patients that did not respond to sapropterin treatment.

The applicant's rationale to conduct this study as an open label, non-blinded study was accepted as blinding would not be possible due to the hypersensitivity reactions triggered by pegvaliase administration, particularly during dose the induction and titration phases. Due to the lack of a placebo group some form of bias cannot be excluded, especially pertaining to the clinical efficacy endpoints (e.g., protein intake and neurocognitive /neuropsychiatric endpoints). Nevertheless, the lack of a placebo was considered acceptable as the clinical efficacy endpoints in this study were exploratory in nature.

Study 165-302 part 1

Patients who participated in previous studies (phase II studies and 165-301) were eligible for inclusion in part 1 of study 165-302 if the patients had been on a stable randomised pegvaliase dose (20 or 40 mg/day) for at least 2 weeks in study. Most patients in the phase II studies were titrated based on phenylalanine levels (≤600 µmol/L) and tolerability which is in line with the treatment strategy in clinical practice. 152 patients who completed the maintenance phase in study 165-301 were included in part 1 of study 165-302. As the minimum duration to reach maintenance was 9 weeks and 25 weeks at maximum, patients who entered part 1 of study 302 received an additional 13 weeks of maintenance treatment. Patients who progressed up to end part 3 (n=82) had a maintenance duration of 36 to 52 weeks when entering part 4.

At the start of part 1 of the study patients received the VS as pharmaceutical form, however during the study part PFS was introduced as new pharmaceutical form. A total of 52 patients were on 20 mg pegvaliase PFS, 56 patients on 40 mg pegvaliase PFS, 53 patients on 20 mg pegvaliase VS and 50 patients on 40 mg pegvaliase VS completed study 165-301. Due to the early closure of study 165-301 only few patients who achieved the target dose using the PFS were transitioned to part 1 of study 165-302 (n=11). The majority of the patients using the PFS were transitioned to part 4 of study 165-302 which is the same as the product to be commercialised.

Study 165-302 part 2

The randomized discontinuation (RDT), placebo-controlled design in Part 2 of Study 165-302 was chosen principally to demonstrate the effect of the 8 weeks withdrawal of pegvaliase on previously controlled blood phenylalanine levels. This design was appropriate to show the short-term effects of the withdrawal of pegvaliase treatment on blood phenylalanine levels, as it was known from previous studies that blood phenylalanine levels would change rapidly.

The patient population to be enrolled in part 2 of study 165-302 was enriched with patients who had ≥20% reduction of phenylalanine from baseline study 165-301 (n=77) or preceding phase II study PAL-003 or Study 165-205 (n=9). This was the mITT population.

As a secondary endpoint the applicant looked at neurocognitive/neuropsychiatric scores. However, the CHMP questioned whether a relevant clinical effect on these scores could be observed in this short study period. In this respect part 4 of study 165-302 is considered to more relevant for assessing the effect of treatment on those outcomes.

Study 165-302 part 4

Part 4 is an ongoing open-label long-term follow part study. 190 patients from Study 165-301 were included in this part, meaning that 7.2% (n=71) of the patients discontinued during the clinical program for various reasons. It should be noted that patients who discontinued where not considered in the primary analyses. These analyses included measurements of patients up to 14 days of the last dose received (regardless of whether this was the end of the study).

Neurocognitive/neuropsychiatric symptom scores were collected in this part of the study. Patients who were either on 20 mg/day or 40 mg/day and did not achieve a target blood phenylalanine level of ≤600 µmol/L could be titrated to 40 or 60 mg/day.

Efficacy data and additional analyses

Study 165-301

Treatment with pegvaliase gradually reduced mean blood Phe concentration for both the 20 mg/day and the 40 mg/day randomized dose groups and mean blood Phe concentration decreases wer greater in the 40 mg/day compared to the 20 mg/day.

The primary analysis using the MMRM resulted in differences in LS means (95% CI)of -923.25 (-1135.04, -711.46) µmol/l (p<0.0001) for the pooled active vs. the 20 mg/day placebo groups and -638.27 (-858.97, -417.57) µmol/l (p<0.0001) for the pooled active vs. the 40 mg/day placebo groups.

It is difficult to conclude on the magnitude of the effect of pegvaliase on Phe levels as most patients either discontinued or transitioned to Study 165-302 when the target dose was reached.

Study 165-302 part 2

The difference from baseline in blood Phe concentrations between patients randomized to active group (pooled) and the placebo groups (20 mg/day and 40 mg/day) was statistically significant (P<0.0001). This comparison was done as placebo groups were not comparable according to the study protocol.

Comparisons of the LS mean differences in change from blood Phe concentration (95% CI) from the start of Part 2 (baseline) to the end of Part 2 (Week 8) showed that the difference in LS means was -923.25 µmol/L and statistically significant between the pooled active group versus the 20 mg/day placebo group (-1135.04 to -711.46; P < 0.0001). Additionally, the difference in LS means between the pooled active group versus the 40 mg/day placebo group was -638.27 µmol/L and was also statistically significant (-858.97 to -417.57; P < 0.0001).

Analysis of the difference in other secondary efficacy endpoints (ADHD-RS IV Inattention Subscale for all subjects, PKU POMS Confusion Subscale, PKU POMS TMD, and POMS TMD) did not show significant differences in symptoms between subjects who remained on pegvaliase versus those who were administered placebo during Part 2. This was expected due to the short duration of this phase of the study.

Study 165-302 Part 4

In part 4 of the study, at database lock 23 September 2016 (week 41) 65.3% of the patients achieved a blood phenylalanine level ≤600 µmol/L after treatment was initiated in study 165-301. After 30 months of total pegvaliase treatment from baseline study 165-301, 73% of patients achieved a blood phenylalanine level ≤600 µmol/L (data cut-off 5 February 2018).

The median change from baseline part 4 up to week 41 for blood phenylalanine levels for the difference dosing categories was -1109.0 µmol/L in the <20 mg/day (n=38), -1126 µmol/L in the 20 -<40 mg/day

(n=112), -761 in the 40-<60 mg/day (n=181) and -516 in the ≥60 mg/day (n=69) group. These reductions were sustained up to 18 months of pegvaliase treatment. After 30 months total treatment from baseline study 165-301 73% of the patients showed a response. As a stopping rule it is stated, in SmPC section 4.4, that both the physician and patient need to reconsider pegvaliase treatment if after 18 months no satisfactory response is observed. Depending on other beneficial effects observed, the physician/patient may decide to continue treatment.

From part 4 baseline to week 41, only numerical changes in ADHD Inattention Score for the difference dosing categories (e.g. <20 mg/day, ≥20 -<40 mg/day (n=130), ≥40-<60 mg/day (n=185) and ≥60 mg/day (n=69)) were observed. For the PKU-POMS a similar trend is observed.

Based on published literature (Spencer 2010 et al) a 5.2 points difference for ADHD-RS is considered the minimal important clinical difference (MICD). Analysis showed that after 18 months of treatment the MICD of 5.2 points was exceeded. In patients who are more symptomatic (ADHD-RS IA with baseline score of >9) this MCID was exceeded after 12 months of treatment.

An analysis of ADHD inattention and PKU POMS confusion subscales by change in blood phenylalanine from baseline quartiles, showed that patients with the largest phenylalanine reductions (Quartile 1) experienced the greatest improvements in ADHD inattention and PKU POMS confusion subscales.

In this part of the study, dose could be increased to 60 mg/day in subjects that had had more than 52 weeks of pegvaliase treatment (combined duration of therapy in 165-302 and previous studies), and a minimum of 8 weeks of treatment at 40 mg/day.

Substantial reduction in blood phenylalanine was observed after dose increase to 60 mg/day. In subjects who were titrated to 60 mg/day in 165-302 and maintained that dose for at least 4 weeks with at least 80% compliance (60 mg/day stable cohort), the mean (SD) blood phenylalanine levels were reduced from 1083.7 (370.53) µmol/l to 700.3 (546.25) µmol/l at 16 weeks and to 560.9 (488.39) µmol/l at 32 weeks after dose increase. Reduction in blood phenylalanine was maintained through 72 weeks of dosing.

For this reason the maximum recommended dose of pegvaliase is 60mg daily in the maintenance phase of treatment. This follows an induction phase, with a recommended starting dosage of pegvaliase of 2.5 mg administered once per week for 4 weeks in line with the induction phase of Study 165-301. The dosage should then be escalated gradually based on tolerability to the daily maintenance dosage required to achieve blood phenylalanine level of 120 to 600 µmol/l. The maintenance dosage is individualised to achieve patient's blood phenylalanine control (i.e., a phenylalanine level between 120 to 600 µmol/l) taking into account patient tolerability to pegvlaiase and dietary protein intake.

PAL-003

Data up to 3.5 years of follow-up showed that under continued pegvaliase treatment patients show a further decrease of blood phenylalanine levels and numerical improvement in the neurocognitive/ neuropsychiatric symptoms scores.

Additional analyses

Overall treatment experience from Study 301 and Study 302

At the time of the data cut off, 188 out of the 261 patients received treatment for at least 1 year, 4 patients completed treatment, and 69 discontinued treatment in the first year. Of these 188 patients, 164 patients received treatment for at least 2 years, 2 patients continued treatment but had not yet reached 2 years of treatment, and 22 patients discontinued in the second year, and 9 patients discontinued after 2 years of treatment. Of the 100 patients who discontinued treatment, 40 patients discontinued due to an adverse event, 29 patients discontinued due to patient decision, 10 patients discontinued due to physician

decision, and 21 patients discontinued to other reasons (e.g. lost to follow up, pregnancy, or protocol deviation).

Mean blood phenylalanine levels reduced from 1233 µmol/l at baseline to 565 micromol/l at Month 12 (Nn=164) and 345 µmol/l at Month 24 (Nn =90), and these reductions in mean blood phenylalanine levels were maintained through Month 36 (341 µmol/l; Nn =48) (see Table 4 and Figure 1). Median change from baseline was 634 micromol/l at Month 12, 965 micromol/l at Month 24, and 913 µmol/l at Month 36.

The data indicate that the reduction of phenylalanine after 12 months of pegvaliase treatment in the post-hoc defined 3rd Line Subpopulation does not significantly differ from the I/T/M or sapropterin non-responders irrespective of diet. Similar results were obtained after 24 months of treatment.

Considering that some patients in this condition can potentially be managed by a restricted diet, it was proposed to modify the indication applied by the applicant by removing reference to sapropterin to ensure that pegvaliase would only be used in patients who have exhausted all other means to control their phenylalanine blood levels. Therefore, the CHMP considered that the indication for pegvaliase should be for the treatment patients with PKU and uncontrolled blood phenylalanine levels >600 µmol/L despite prior management with available treatment options.

Assessment of paediatric data on clinical efficacy

Only twelve paediatric patients aged 16 to 18 years were included in the clinical programme (efficacy data was available for 11 patients and safety data for all 12 patients). After 36 months continued treatment 8 out 12 patients reached blood phenylalanine levels ≤600 µmol/L. No consistent differences in antibody titers or adverse events incidences between the paediatric group and the adult group were observed. Therefore the indication for pegvaliase includes patients from 16 years of age.

2.5.4. Conclusions on the clinical efficacy

The clinical efficacy of pegvaliase is mainly based on a pharmacodynamic effect which has been convincingly demonstrated. The data, in patients aged 16 years and older, demonstrate that in the maintenance phase under continued pegvaliase treatment blood phenylalanine levels reduced to target levels of ≤600 µmol/L in the majority of patients. This effect appears to be sustained in most patients as demonstrated in the longer term studies. Based on the knowledge of the natural history of the disease this is considered an important effect.

Even though the effects of treatment on other reported outcomes are more difficult to confirm as they are mainly based on uncontrolled data, available evidence also suggest that with pegvaliase treatment patients showed some improvement in the neurocognitive/neuropsychiatric symptoms and were capable of increasing their protein intake from intact foods, and increase the intake of phenylalanine.

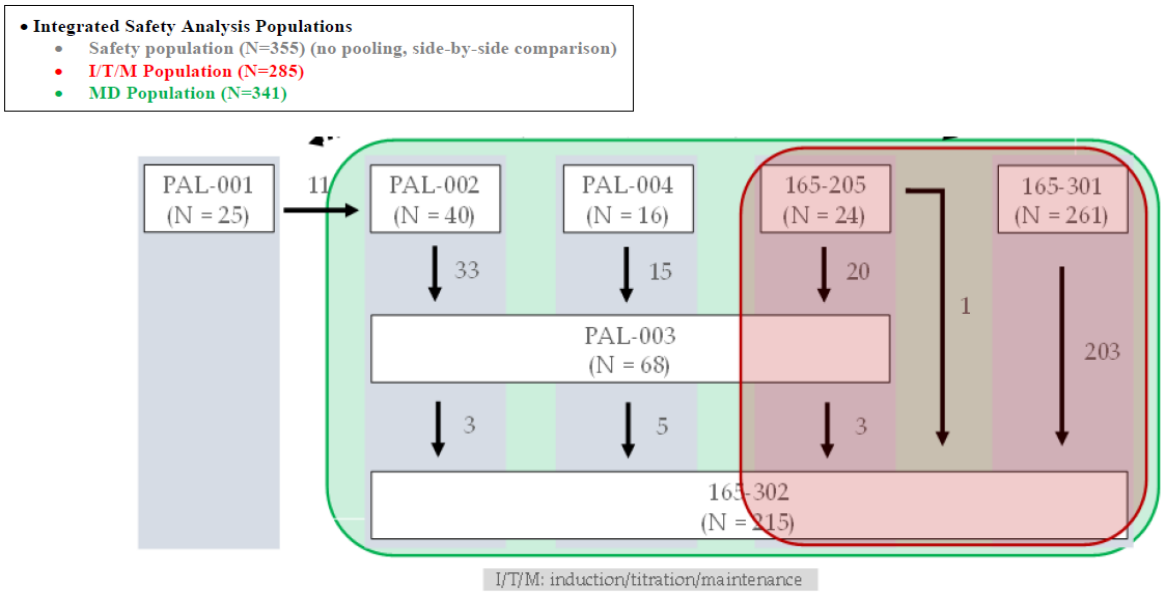
Overall, it was concluded that pegvaliase is an effective treatment of patients with phenylketonuria who have inadequate blood phenylalanine control.

2.6. Clinical safety

Patient exposure

A number of different populations were defined for the safety analysis (**Figure 22**).

Figure 22. Overview of Pooling of Patients for Integrated Safety Analyses by Parent Study and Analysis Population



Parent study was the study in which pegvaliase was first administered (PAL-001, PAL-002, PAL-004, 165-205, and 165-301).

Up to the data cut-off date (5 February 2018), 355 patients were exposed to at least one injection of pegvaliase (safety population). Patients exposed to multiple doses of pegvaliase were pooled in the multiple dose (MD) population (n=341). Duration of treatment per study is summarised in **Table 24**.

Table 24. Pegvaliase Dosing Across Studies

Study ID (Phase)	Study Period/Parts	Pegvaliase Dosing	Treatment Duration
PAL-001 (Phase 1)		Single dose of 0.001, 0.003, 0.01, 0.03 or 0.1 mg/kg (VS)	Single injection
PAL-002 (Phase II)	**Part 1**	Fixed doses: 0.001, 0.003, 0.01, 0.03 or 0.1 mg/kg/week (VS)	For 8 weeks
	Part 2	Adjustable dosing: Same as at completion of Part 1 with a maximum of 5.0 mg/kg/week to reach target blood phenylalanine of 60-600 µmol/L (VS)	For 8 weeks
PAL-004 (Phase II)		Adjustable daily dosing (defined as 5 days/week for this study) based on safety and efficacy (VS)	13 weeks
165-205 (Phase II)	**Induction**	Fixed dose of 2.5 mg/week (VS)	4-8 weeks
	Titration	Gradual upward titration to a maximum dose of 375 mg/week (VS)	At least 4 weeks
	Maintenance	Target dose at which patient sustained blood phenylalanine reduction of ≤ 600 µmol/L; maximum allowed dose of 75 mg/day (375 mg/week) (VS)	8 -15 weeks
PAL-003 (Phase II Extension)		0.001 mg/kg to a maximum of 5.0 mg/kg/week or 375 mg/week (dosing frequency once a week to daily) (VS; PFS)	Up to 102 months (Ongoing)
165-301 (Phase III)	**Induction**	Fixed dose of 2.5 mg/week (VS; PFS)	4 weeks
	Titration	Upward titration from 5 mg/week up to the randomized daily dose of 20 mg/day or 40 mg/day (VS; PFS)	Up to 30 weeks
	Maintenance	20 mg/day or 40 mg/day daily dosing (VS; PFS)	At least 2 weeks

165-302 (Phase III)	**Part 1**	20 mg/day or 40 mg/day (VS)	3 -13 weeks
	Part 2 (RDT)	20 mg/day or 40 mg/day, or Placebo (VS)	8 weeks
	Part 3	20 mg/day or 40 mg/day -one-way crossover study to compare the PK and PD of two different drug presentations (VS versus PFS) of pegvaliase	6 weeks
	Part 4	40 mg/day, or the dose at the end of PAL-003 (10, 20, 40 or 60 mg/day). Maximum of 60 mg/day (PFS)	Up to 212 weeks (Ongoing)

The I/T/M Population which pooled data from patients from parent studies 165-205 and 165-301 and where the induction, titration, and maintenance treatment regimen was used, n=285) is considered to be the most clinically relevant. All of these patients received induction treatment (18.4 person-years of total treatment exposure), 273 patients underwent titration (269.3 person-years) and 175 patients received maintenance treatment (409.3 person-years).

In the I/T/M Population, 73.3% (209/285) have had at least 1 year of exposure, 63.5% (181/285) have had at least 2 years of pegvaliase exposure, 40.7% (116/285) have had at least 3 years of pegvaliase exposure, and 18.9% (54/285) have had at least 4 years of exposure (**Table 25**). Exposure by dose level in this population is summarised in **Table 26**.

Table 25. Duration of Exposure to pegvaliase (data cut-off:05 Feb 2018)

Subjects by duration of treatment, n (%)	**I/T/M Population (N=285)**	**MD Population (N=341)**
≥ 6 months	229 (80.4%)	275 (80.6%)
≥ 1 year	209 (73.3%)	254 (74.5%)
≥ 2 years	181 (63.5%)	221 (64.8%)
≥ 3 years	116 (40.7%)	152 (44.6%)
≥ 4 years	54 (18.9%)	87 (25.5%)
Total treatment exposure (person-years) [a]	**701.6**	**952.6**

[a] *The duration in months was calculated from the first dose to the last dose administered across all studies in which a subject was enrolled. Intervals of missing doses that were > 28 consecutive days were excluded from the calculation of treatment duration.*

Table 26. Exposure to pegvaliase by dose Level (I/T/M Population)

Dose Level Range [a]	**Number of Subjects**	**Treatment Exposure (Person-Years) [b]**
Placebo [c]	28	4.4
> 0 and < 20 mg/day	285	142.5
⩾ 20 and < 40 mg/day	257	160.0
⩾ 40 and < 60	222	303.1
⩾ 60 mg/day	96	91.5
Total	**285**	**701.6**

[a] *Each dose level group included all subjects who received a daily dose of pegvaliase within the specified dose range. Subjects could have been included in more than one dose level group.*

[b] *Total treatment exposure was aggregated duration of treatment across all subjects (for each subject, time from the first dose to the last dose administered across all studies in which the subject was enrolled). Intervals of missing doses that were >28 consecutive days were excluded from the calculation of treatment duration.*

[c] *Includes subjects who received placebo during Part 2 of 165-302.*

Adverse events

All patients in the I/T/M Population experienced at least 1 drug-related Adverse Event (AE) (**Table 27**). Assessment of an AE as being drug related and this was determined at the investigators discretion based on "reasonable possibility" (e.g. temporal relationship between AE occurrence and pegvaliase dose, presence of other pre-disposing factors).

Table 27. Adverse events assessed by the investigator as related to study drug in >10% of the patients per treatment phase (I/T/M population)

Number of Subjects with Event[h] (%)	Induction (I) (N=285)	Titration (T) (N=273)	Induction/ Titration (I/T) (N=285)	Maintenance (M) (N=175)	Overall I/T/M (N=285)
Total treatment exposure (person-years)	18.4	269.3	292.1	409.3	701.6
Blood and lymphatic system disorders					
Lymphadenopathy	3 (1.1%)	26 (9.5%)	28 (9.8%)	21 (12.0%)	41 (14.4%)
General disorders and administration site conditions					
Injection site reaction [a]	205 (71.9%)	233 (85.3%)	256 (89.8%)	112 (64.0%)	266 (93.3%)
Immune system disorders					
Hypersensitivity reactions [b]	58 (20.4%)	158 (57.9%)	184 (64.6%)	101 (57.7%)	213 (74.7%)
Acute systemic hypersensitivity reaction	0	13 (4.8%)	13 (4.6%)	3 (1.7%)	16 (5.6%)
Angioedema	2 (0.7%)	14 (5.1%)	16 (5.6%)	5 (2.9%)	21 (7.4%)
Serum sickness-like reaction (modified)	4 (1.4%) 4 (0.22)	2 (0.7%)	6 (2.1%)	1 (0.6%)	7 (2.5%)
Complement factor C3 decreased [c]	14 (4.9%)	186 (68.1%)	189 (66.3%)	127 (72.6%)	206 (72.3%)
Complement factor C4 decreased [c]	7 (2.5%) 7 (0.38)	180 (65.9%)	182 (63.9%)	62 (35.4%)	192 (67.4%)
High sensitivity CRP levels increased [d]	0	47 (17.2%)	47 (16.5%)	16 (9.1%)	59 (20.7%)
Investigations					
Hypophenylalaninemiae	0	43 (15.8%)	43 (15.1%)	107 (61.1%)	125 (43.9%)
Gastrointestinal disorders					
Nausea	24 (8.4%)	57 (20.9%)	71 (24.9%)	48 (27.4%)	100 (35.1%)
Abdominal painf	18 (6.3%)	40 (14.7%)	53 (18.6%)	47 (26.9%)	89 (31.2%)
Vomiting	9 (3.2%)	48 (17.6%)	53 (18.6%)	43 (24.6%)	86 (30.2%)
Musculoskeletal and connective tissue disorders					
Arthralgia [g]	107 (37.5%)	206 (75.5%)	223 (78.2%)	109 (62.3%)	241 (84.6%)
Myalgia	7 (2.5%)	24 (8.8%)	31 (10.9%)	19 (10.9%)	45 (15.8%)
Joint swelling	3 (1.1%)	14 (5.1%)	17 (6.0%)	6 (3.4%)	23 (8.1%)
Joint stiffness	2 (0.7%)	16 (5.9%)	18 (6.3%)	4 (2.3%)	22 (7.7%)
Musculoskeletal stiffness	2 (0.7%)	11 (4.0%)	12 (4.2%)	9 (5.1%)	20 (7.0%)
Nervous system disorders					
Headache	49 (17.2%)	95 (34.8%)	119 (41.8%)	81 (46.3%)	156 (54.7%)
Respiratory. thoracic and mediastinal disorders					
Cough	1 (0.4%)	53 (19.4%)	54 (18.9%)	36 (20.6%)	83 (29.1%)
Skin and subcutaneous tissue disorders					
Rash	24 (8.4%)	80 (29.3%)	95 (33.3%)	41 (23.4%)	111 (38.9%)
Urticaria	10 (3.5%)	65 (23.8%)	71 (24.9%)	37 (21.1%)	87 (30.5%)
Erythema	3 (1.1%)	29 (10.6%)	32 (11.2%)	10 (5.7%)	38 (13.3%)
Pruritus	14 (4.9%)	63 (23.1%)	71 (24.9%)	38 (21.7%)	91 (31.9%)
Skin exfoliation	1 (0.4%)	0	1 (0.4%)	3 (1.7%)	4 (1.4%)
Maculo-papular Rash	2 (0.7%)	8 (2.9%)	10 (3.5%)	5 (2.9%)	13 (4.6%)
Alopecia	0	19 (7.0%)	19 (6.7%)	39 (22.3%)	52 (18.2%)

a Reflect all terms reported under the MedDRA high level term Injection Site Reactions
b Hypersensitivity reaction were identified using hypersensitivity modified narrow SMQ with acute systemic hypersensitivity reaction (NIAID/FAAN episodes as adjudicated by external expert). Injection site rash and injection site urticaria were excluded from the hypersensitivity narrow SMQ.
c Complement factor C3/C4 decrease is defined as changing from normal or high complement baseline value to low post-baseline value

d Reflects High sensitivity C-Reactive Protein (hsCRP) levels above upper limit of normal (>0.287 mg/dL) over a 6 month period. hsCRP was measured in a total of (N=261) subjects
e Hypophenylalaninaemia event is defined as the period with at least 2 consecutive blood phenylalanine concentrations <30 umol/L.
f Abdominal pain reflects the following preferred terms: abdominal pain, abdominal pain upper and abdominal discomfort.
g Arthralgia reflects the following preferred terms: arthralgia, back pain, musculoskeletal pain, pain in extremity and neck pain.
h the number of patients in each treatment phase do not add up to the totality of patients in the I/T/M as patients could have adverse events reported in each phase.

The incidence and event rates for the most commonly reported AEs in the MD Population were generally consistent with what was reported in the I/T/M Population (data not shown).

The overall exposure-adjusted AE rate in the I/T/M Population was lower at higher dose levels, with rates of 42.77, 30.67, 20.37 and 15.44 AEs/person-year for the <20, ≥20 to <40, 40 to <60, and ≥60 mg/day dose levels, respectively. The rate of AEs assessed by the investigator as study drug-related also declined as the dose level increased (32.54, 20.51, 10.81, and 6.95 AEs/person-year for the <20, ≥20 to <40, ≥40 to <60, and ≥60 mg/day dose levels, respectively).

Serious adverse event/deaths/other significant events

Serious adverse events

A total of 91 SAEs were reported in 64 subjects in the ITM Population (78 in the MD population) during the studies (**Table 28**). Of these, a total of 42 (46.2%) SAEs were assessed treatment related by the investigators, 87 (95.6%) SAEs resolved, 1 (1.1%) SAE resolved with sequelae, and 18 (19.8%) SAEs led to study drug or study discontinuation.

Table 28. Serious Adverse Events by Preferred Term in the I/T/M and MD Population.

Number of Patients with Event (%)	**I/T/M (N=285)**	**Multiple Dose (N=341)**
Total treatment exposure (person-years)c	579,6	809,7
Any Serious AEs [a]	**64 (22,4%)**	**78 (22,9%)**
Anaphylactic reaction	14 (4,9%)	15 (4,4%)
Hypersensitivity [a]	9 (3,2%)	10 (2,9%)
Blood creatine phosphokinase increased	5 (1,8%)	5 (1,5%)
Anaphylactoid reaction	3 (1,1%)	3 (0,9%)
Anxiety	3 (1,1%)	3 (0,9%)
Angioedema	1 (0,4%)	2 (0,6%)
Appendicitis	2 (0,7%)	2 (0,6%)
Arthralgia	1 (0,4%)	2 (0,6%)
Asthma	1 (0,4%)	2 (0,6%)
Chest pain	2 (0,7%)	2 (0,6%)
Depression	1 (0,4%)	2 (0,6%)
Non-cardiac chest pain	2 (0,7%)	2 (0,6%)
Road traffic accident	2 (0,7%)	2 (0,6%)
Serum sickness	2 (0,7%)	2 (0,6%)
urticaria [a]		1 (0,3%)
swollen tonque [a]	1 (0,4%)	1 (0,3%)
pseudomonas infection[a]	1 (0,4%)	1 (0,3%)

[a] *Additional Adverse Events Upgraded to Serious due to Medical Significance by the Sponsor in the Safety Database are included in this table.*

The most frequently reported SAE reported in patients were anaphylactic reactions. Anaphylactic/anaphylactoid reactions led to withdrawal of the drug in half of the patients and to dose interruptions/dose reductions in the other half of the patients. Blood CPK increase was reported by five patients. Due to the occurrence of other predisposing factors (e.g. starting new exercise regimens), this SAE was assessed as unrelated to pegvaliase.

Deaths

Across the pegvaliase clinical studies, one death due to accidental electrocution was reported in the Phase III study, 165-301. This event was assessed as unrelated to the study drug which is acceptable.

Other significant events

Immunological events

As a foreign derived therapeutic protein, pegvaliase has immunogenic potential. Hypersensitivity events were reported in the pegvaliase clinical studies by 93.7% of patients in the I/T/M Population, with most patients experiencing mild (Grade 1: 18.2%) or moderate (Grade 2: 62.5%). In cases were HAEs lead to permanent drug withdrawal this was often due to a combination of recurrent multiple mild to moderate events.
The most commonly reported HAEs (by PT) in the I/T/M Population were injection site reactions (93.3%), arthralgia (84.6%), hypersensitivity reactions (74.7%), headache (54.7% rash (38.9%),%, nausea (35.1%), pruritus (31.9%) and urticaria (30.5%).. HAEs occurred most frequently in the first 6 months of treatment and peaked after three weeks of treatment (part of the induction phase). HAEs were dose-independent. The incidence of HAE episodes in patients receiving placebo doses was 14.3%.

Acute systemic hypersensitivity reactions, consistent with the NIAID/FAAN clinical diagnostic criteria for likely anaphylaxis, were the most clinically important identified risks in the pegvaliase development program. Using the NIAID/FAAN clinical criteria, potential episodes of acute systemic hypersensitivity reactions were evaluated by an independent expert (allergist/immunologist).

In the I/T/M population, 16 patients experienced 25 acute systemic hypersensitivity reactions. In 11 of the 25 events, epinephrine was administered. The incidence was highest, but not exclusive to the first 6 months of treatment. No clear dose-relationship was observed. Following a request from the CHMP the applicant aimed to identify risk factors for ASHRs. No relationship with age, gender, baseline phenylalanine, cumulative dose, time from last dose to events, last blood phenylalanine before event or concomitant medication was identified (**Table 29**).

Table 29. Profile of Acute Systemic Hypersensitivity Reactions in the I/T/M population

	Number of Subjects with Events (N=16)
Number of Subjects with at least one event, n (%)	16 (100)
Number of Events, n	25
Cumulative dose level at the time of first event (mg) [a]	
n	16
Mean (SD)	7352,1 (13040,8)
Median	2507,7
Min, Max	95, 48455
Time from last dose escalation to event onset (days) [b]	

n	25
Mean (SD)	82,5 (126,6)
Median	36
Min, Max	0, 451
Last blood Phe Level before event (umol/L) [b]	
n	25
Mean (SD)	1032,2 (335,3)
Median	980
Min, Max	9, 1631

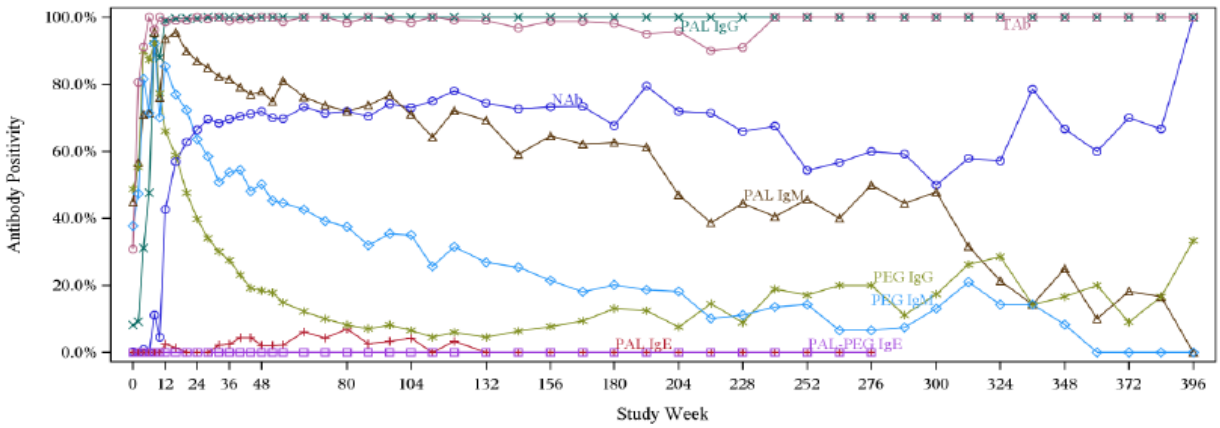
Due to the occurrence of the ASHRs, a protocol amendment with risk minimization measures (RMMs) was instated that required pre-treatment with H1 antagonist, H2 antagonist, and antipyretic medications before the injections. In addition, a trained observer should be present the first 16 weeks of treatment and an epi-pen should be carried by the patient. The amount of discontinuations decreased after the instatement of the RMM (from 23.8% to 13.6% in the first 6 months and from 32.9% to 18.6% in the first year).

There were 2 subjects in the PAL-001 single dose study, excluded from the MD and I/T/M Populations, who were concurrently using another PEGylated injectable, medroxyprogesterone (Depo-Provera), at the time of their pegvaliase dose. These 2 subjects experienced a total of 3 episodes of hypersensitivity reactions, associated with the use of medroxyprogesterone, of which 1 episode in 1 subject was an acute systemic hypersensitivity reaction meeting the NIAID/FAAN criteria.

Immunogenicity

All patients treated with pegvaliase developed anti-drug antibodies (ADA). Overall, the pattern of antibody development was generally similar across all studies in the pegvaliase clinical program, regardless of the dosing schedule during the induction/titration period. All patients developed sustained antibodies against the phenylalanine ammonia lyase (PAL) protein, and the majority of patients developed transient antibodies against polyethylene glycol (PEG) (**Figure 23**).

Figure 23. Incidence of antibody positivity over time in patients in the MD population



Number of Subjects

NAb	337	293	253	240	217	235	178	156	131	99	64	44	35	30	22	14	12	10	3
PAL IgE	78	83	46	40	49	43	23	26	16	7	7	3	1	2	0	0	0	0	0
PAL IgG	339	295	254	240	217	230	179	154	130	98	66	44	34	30	23	14	12	11	3
PAL IgM	339	294	253	242	217	235	180	156	130	99	66	45	35	30	23	14	12	11	3
PAL-PEG IgE	78	83	46	40	49	43	23	26	16	7	7	3	1	2	0	0	0	0	0
PEG IgG	339	295	254	240	217	233	180	155	130	99	66	45	35	30	23	14	12	11	3
PEG IgM	339	294	253	242	217	235	180	156	130	99	66	45	35	30	23	14	12	11	3
TAb	260	219	193	185	158	179	129	107	84	54	24	11	9	7	5	2	2	2	1

Data demonstrated an association between antibody response and adverse events. Typically, hypersensitivity adverse events occurred most frequently during the first six months of treatment when the early immune response comprised of PEG IgM, PEG IgG and PAL IgM responses peaked, C3/C4 levels declined, and CIC levels were at their highest. The frequency of HAEs decreased over time in long term treatment as the incidence of these antibodies decreased. With prolonged treatment, C3/C4 levels did not return to baseline for the patients in the highest quartiles. Additional analysis provided by the applicant compared the incidences of AE of interest in patients with complement C3 and C4 < lower limit of normal (LLN) and >LLN. Safety data was available from 180 subjects with greater than 1 year of data and 159 subjects with greater than 2 years of data. There was a trend observed for higher incidences of lymphadenopathy for those subjects with low C3 (7.6% vs 2.7%) but the same trend was not seen with low C4 (4.8% vs 5.8%). No significant differences were found in the other AE categories.

Since complement C3 and C4 are an essential part of the innate immune system, it was of concern that long term suppression of complement levels might suppress the immune system. However, no clear difference was observed between patients with C3 < LLN and > LLN; 17.1% and 16.0% of the patients respectively (infections and infestations >30 days) for the first year and 17.3% vs 15.5% for the second year.

In general, an association between ADA titres and frequency of hypersensitivity reactions and injection site reactions was observed for some antibody analytes, such that patients who had lower mean titres experienced fewer hypersensitivity reactions and injection site reactions. Patients who experienced anaphylaxis events had higher mean antibody titres for some analytes than patients that did not experience anaphylaxis, and ADA titres at the time of an anaphylaxis event tended to be higher than the mean over the entire study. However, individual titres overlapped between patients who experienced anaphylaxis events and those who did not; as a result, no specific antibody titres could be identified as predictive of an HAE or anaphylactic event.

Hypophenylalaninaemia

In the I/T/M Population, 125 (43.9%) patients met the definition of hypophenylalaninaemia (at least 2 consecutive blood phenylalanine levels <30 µmol/L). In 80% of the patients, phenylalanine levels dropped below 5 µmol/L (limit of detection of the assay). The median time to onset from the first dose to the first hypophenylalaninaemia event was 393 days (range 51 to 1,405 days) and the median duration of hypophenylalaninaemia events was 161 days (range 35 to 1,408 days). Measures were included in the protocol to prevent hypophenylalaninaemia (regular blood phenylalanine measurements, possibility for dose decrease and consultation of a dietician to increase protein intake).

In the I/T/M Population, incidences for most of the AE categories were lower in the hypophenylalaninaemia group compared to the group of patients that did not experience hypophenylalaninaemia including overall AEs (96 % vs 100%), investigator assessed treatment-related AEs (81.6% vs 100%), SAEs (8.8% vs 23.1%) and CTCAE ≥ Grade 3 events (8.8% vs 28.1%). Only the PTs of ‘alopecia’ and ‘amino acid level decreased’ had both higher patient incidence and event rates in the hypophenylalaninaemia group.

Angioedema

A total of 21 (7.4%) subjects experienced 37 episodes of angioedema in the I/T/M Population with a rate of 0.06 episodes/person-year. Within 37 episodes of angioedema, 43 AE PTs were reported. All reported episodes of angioedema were CTCAE Grade 1 (31 episodes in 16 [5.6%] subjects) or Grade 2 (6 episodes in 5 [1.8%] subjects) and there were no Grade 3 or 4 AEs (Table 8.13.24). The most frequently reported AE PTs associated with angioedema episodes in the I/T/M Population were swelling face (2.5%) swollen tongue (1.8%), lip swelling (1.1%), and pharyngeal oedema (1.1%).

Of the 21 subjects who reported angioedema episodes in the I/T/M Population, 11 (3.9%) subjects reported while on pegvaliase dose levels of > 0 to < 20 mg/day, 9 (3.5%) subjects on ≥ 20 to < 40 mg/day and the remaining 3 (1.4%) on ≥ 40 to < 60 mg/day.

In the I/T/M Population, the mean (SD) time to onset of the angioedema episode from the most recent pegvaliase dose was 26.8 (114.90) hours with a median (range) time to onset of 0 (0 [i.e. immediately following dosing] to 696) hours. Of the 37 episodes of angioedema, 29 occurred on the same day of dosing, 5 occurred within 1 day after the last dose, and 1 episode each was reported 3 and 4 days, respectively, after the last dose; in addition one subject experienced an episode (reported AE of swollen tongue, that lasted for a day, for which the dosing was interrupted) 29 days (696 hours) after last dose received.

Serum Sickness-Like Reactions

In the I/T/M Population, using the broadest search strategy (PTs of serum sickness and serum sickness-like reaction plus a symptomatic search), 9 episodes of serum sickness with 13 events were reported in 9 subjects (3.2%; 0.02 AEs/person-year).

Seven of the 9 episodes were reported PTs of serum sickness; the remaining two episodes in two subjects included events of arthralgia and pyrexia with either rash or allergic dermatitis.

In the I/T/M Population, using the modified search strategy (which focused on the actual reported PTs of serum sickness or serum sickness-like reaction omitting the symptomatic search), 7 AEs in 7 subjects with reported PTs of serum sickness were identified (2.5%; 0.01 AEs/person-year). No additional subject reported serum sickness in the MD Population.

Of the 7 subjects who reported serum sickness in the I/T/M Population, 6 (2.1%; 0.05 AEs/person-year) subjects reported while on pegvaliase dose levels of > 0 to < 20 mg/day, and the remaining 1 (1.4%; 0.01 AEs/person-year) subject on ≥ 40 to < 60 mg/day.

Of the 7 subjects who reported serum sickness in the I/T/M Population, 4 subjects experienced Grade 2 AEs (1.4%), and 3 subjects experienced Grade 3 AEs (1.1%), which resulted in treatment discontinuation for 2 subjects. Both of these AEs were reported as SAEs; the remaining 5 episodes were non-serious events. All 7 AEs of serum sickness resolved without sequelae and had a duration of 1 to 8 days. Of the 7 subjects who reported serum sickness, 2 subjects discontinued treatment and 5 subjects continued treatment without experiencing a recurrence; serum sickness in these 5 subjects was managed with drug interruption, dose reduction, and/or concomitant medication.

Arthralgia

Of the 285 subjects in the I/T/M Population, 241 (85 %) reported 1,876 AEs of arthralgia (2.67 events per patient-year).

Arthralgia occurred as early as the first dose and also occurred at any time during treatment. Arthralgia was most frequent during Induction and Titration phases (223/285; 78% of subjects; 1264 episodes over a mean treatment period of 12 months likely due to the levels of circulating immune complexes (CIC) being highest during this period and decreased in the Maintenance Phase (62% of patients; 612 episodes over a mean treatment period of 28 months).

The mean duration of arthralgia was 15 days and 78% of arthralgia episodes had a duration of less than 14 days. Arthralgia persisted up to 936 days (1% of arthralgia episodes persisted at least 180 days). Severe arthralgia (severe pain limiting self-care activities of daily living) was experienced in 14 (5%) patients. Arthralgia episodes were managed with concomitant medicinal products (e.g., nonsteroidal anti-inflammatory drugs, glucocorticoids, and/or antipyretic), dosage reduction (4% of episodes),

treatment interruption (4% of episodes), or treatment withdrawal (0.6% of episodes), and 97% of arthralgia episodes resolved by the time of the data cut off.

While the specific location on the body for AEs of arthralgia was not systematically collected, a review of the reported verbatim terms for these events shows that the arthralgia events were widespread but more common in the extremities and back.

Persistent arthralgia (lasting at least 6 months) occurred in 19 (7%) patients with a total of 24 episodes. Persistent arthralgia occurred as early as 6 days and up to 1,526 days into treatment (median: 566 days from treatment initiation). The dose was not changed for 23 (96%) episodes and dose was reduced for 1 (4%) episode. All persistent arthralgia episodes resolved without sequelae.

Injection site reactions

In the I/T/M Population, 266 (93%) subjects experienced 5,009 AEs related to episodes of ISRs. All ISRs were Grade 1 or Grade 2 in severity. The most common ISRs (occurring in at least 10% of subjects) were bruising, erythema, pain, pruritus, rash, swelling, urticaria and induration.

ISRs were most frequent during Induction and Titration Phase (90% of patients; 3,899 episodes over mean treatment duration of 12 months) and decreased in Maintenance Phase (64% of patients; 1110 over a mean treatment period of 28 months).

Injection site reactions occurred as early as the first dose and can occur at any time during treatment. The mean duration of injection site reaction was 9 days, and 91% of injection site reactions had a duration of less than 14 days 0.8% of injections site reactions persisted at least 180 days, and 99% of injection site reactions resolved by the time of the data cut off.

Three ISRs consistent with granulomatous skin lesions were reported (each reaction occurring in one patient): granulomatous dermatitis (occurred 15 months after Palynziq treatment and lasted 16 days), xanthogranuloma (occurred 12 months after initiation of treatment and lasted 21 months), and necrobiosis lipoidica diabeticorum (occurred 12 months after initiation of treatment and lasted 12 months). Necrobiosis lipoidica diabeticorum was treated with steroid injections and complicated by Pseudomonas infection. All of these injection site reactions resolved. One patient reported soft tissue infection associated with mesenteric panniculitis, which resulted in treatment discontinuation

There was an inverse relationship between incidences of ISRs in the I/T/M Population and dose, with the lowest incidences of ISRs occurring in subjects treated at ≥ 60 mg/day pegvaliase (34.4%) and the highest incidences of ISRs occurring in subjects treated at > 0 and < 20 mg/day pegvaliase (85.3%). The incidences of ISRs in subjects receiving placebo doses was 25.0%.

Laboratory findings

Changes in laboratory parameters observed in haematology, clinical chemistry and urinalysis during clinical studies were not considered clinically meaningful or related to pegvaliase.

A summary of the incidence of laboratory parameters of interest is provided in **Table 30**.

Table 30. Incidences of Laboratory Parameters of Interest

Laboratory Measurement	I/T/M Population (N = 285)	MD Population (N = 341)
UACR ≥ 3 mg/mmol on 3 or more consecutive measurements	12 (4.2%)	16 (4.7%)
Hematuria > 3 RBC/hpf OR > ULN on 3 or more consecutive measurements	8 (2.8%)[a]	15 (4.4%) [a]
UACR ≥ 3 mg/mmol AND hematuria > 3 RBC/hpf OR > ULN on 3 or more consecutive measurements	0	1 (0.4%) [a]

Serum creatinine > 30% above baseline OR > ULN on 3 or more consecutive measurements	8 (2.8%)	14 (4.1%)
Hemoglobin < 100 g/L on 3 or more consecutive measurements	1 (0.4%)	2 (0.6%)
AST ≥ 3 × ULN at 3 or more consecutive measurements	0	0
ALT ≥ 3 × ULN at 3 or more consecutive measurements	0	0
Platelets < 100 × 109/L on 3 or more consecutive measurements	1 (0.4%)	1 (0.3%)
hsCRP > 0.287 mg/dL over a 6 month period[c]	48 (16.8%)	50 (14.7%)

a Three patients were misreported and have been included in this table by the assessor for clarity.
c hsCRP was only measured in 165-301 and 165-302, but the denominator used for calculation of the incidences is the I/T/M Population. This was not performed for Phase II studies including 165-205 (N=24).

Safety in special populations

The majority of the patients in the I/T/M population were between 18 and 65 years of age (n=273). Twelve patients were included aged 16-18. No patients >65 years were included in the studies. There was no difference in the safety profile in the 16-18 year group versus the 18-65 year group.

Safety related to drug-drug interactions and other interactions

No formal studies of drug-drug or drug-disease interactions were submitted.

Discontinuation due to adverse events

Overall, 37.5% of the patients discontinued the study drug at least one time during the study. The majority of these discontinuations occurred during the initiation/titration phase (30.9%). Of the total number of discontinuations, 41.1% was due to adverse events.

Overall, 33% of the patients withdrew from the study of which the majority occurred during the initiation/titration phase (27.0%). Of the total study withdrawals, 27.7% was due to adverse events.

The events leading to drug discontinuation or study withdrawal were predominantly hypersensitivity and acute systemic hypersensitivity reactions.

Post marketing experience

Not applicable.

2.6.1. Discussion on clinical safety

Pegvaliase treatment follows an induction/titration/maintenance dosing regimen. Patient are started on 2.5 mg pegvaliase once a week to induce tolerability by desensitising the immune system. This phase is followed by a titration phase during which the dose is increased in a stepwise manner to a dose tolerated by the patients and achieving the optimal clinical effect (preferably a phenylalanine serum level below 600 µmol/L. After the titration phase the treatment enters the stable maintenance phase in which the lowest effective dosage to achieve and maintain blood phenylalanine control is continued.

In the pegvaliase clinical program, 341 patients received multiple doses of pegvaliase, constituting the MD safety population. The I/T/M safety population includes all patients who were administered pegvaliase using the induction, titration and maintenance regimen. This population includes data from subjects first enrolled in the parent studies 165-205 or 165-301 and from the subsequent extension studies to which they transferred (PAL-003 and 165-302). The I/T/M population is considered to be the most clinically relevant. Even though the overall number of patients in the safety population is small according to the ICH

guideline E1 on assessment of clinical safety, given the low incidence of PKU (1 per 10.000 births), it was considered acceptable.

In the I/T/M Population, 73.3% (209/285) have had at least 1 year of exposure, 63.5% (181/285) have had at least 2 years of pegvaliase exposure, 40.7%(116/285) have had at least 3 years of pegvaliase exposure, and 18.9% (54/285) have had at least 4 years of exposure. Thus, a substantial amount of the total study population (209 patients) had at least one year exposure to the study drug which is sufficient according to ICH guidelines.

All patients in the I/T/M Population (N=285) experienced at least 1 drug-related AE. The most common drug-related AEs among patients were injection site reactions (93.3%), arthralgia (84.6%), hypersensitivity reactions (74.7%), headache (54.7% rash (38.9%),%, nausea (35.1%), pruritus (31.9%) and urticaria (30.5%).These adverse events are considered related to the hypersensitivity reactions observed in all patients.

A total of 91 SAEs were reported, of which 42 were assessed as treatment related. Overall it is reassuring that all SAEs resolved. The most commonly reported SAE PTs (>2 events) included anaphylactic reaction, hypersensitivity, anaphylactoid reaction, blood creatine phosphokinase (CPK) increased, and anxiety. The SAE occurred more frequently in the early treatment phase and were dose-independent.

There is limited data on the comparison of AE incidence in the treatment groups versus the placebo group. In part this is due to the study design of a discontinuation trial in which patients were withdrawn from the study drug for 8 weeks in part 2 of study 165-302. All patients had been exposed to the drug before being randomized to the placebo group, so all patients had already had an immune response against pegvaliase. In addition, the total treatment exposure in the placebo group was only a small proportion of the treatment exposure in the pegvaliase dosing groups.

The incidence of all adverse was assessed per treatment phase (induction, titration and maintenance) In general, the incidence of AEs decreased with treatment duration. AEs are generally highest in the induction/titration phase when the immune-response against pegvaliase peaks. The only AE that has a higher incidence in the maintenance phase is hypophenylalaninaemia.

Although the incidence of AEs was relatively high in the lower dose group (between 0 and 20 mg/day), this can be explained since all patients received this dose in the induction/titration phase which is also the phase in which most immune-response related AEs occur. The AEs seem to be dose independent. This is supported by data from part 4 of study 165-302, in which some patients were up-titrated from 40 mg/day to 60 mg/day. This did not lead to a significant increase of AE's one month before versus one month after the dose increase. Doses up to 60 mg/day are thus not associated with an increase in AEs. An integrated analysis of AE profile per dose group for the new data cut-off will be submitted once the final study reports from the ongoing studies PAL-003 and 165-302 are available.

AEs were most commonly mild to moderate (grade 1 [7.0%]) and grade 2 [68.4%]). Grade 3 and 4 AEs were reported by 22.1% and 2.1% respectively. One patient died by electrocution during the study which was assessed as being unrelated to the study drug.

The most frequent AEs were hypersensitivity AEs, occurring in almost all patients (93.7% of all patients in the I/T/M population). The most common (>20% of patients) HAEs in the I/T/M Population were injection site reactions (93.3%), arthralgia (84.6%), hypersensitivity reactions (74.7%), headache (54.7% rash (38.9%),%, nausea (35.1%), pruritus (31.9%), abdominal pain (31.2%), urticaria (30.5%), vomiting (30.2%). Cough (29.1%) Most patients experienced mild (Grade 1: 18.2%) or moderate (Grade 2: 62.5%) events. Most HAEs required no dose modification (91.6%; patients continued to be dosed) and resolved (97.5%). In clinical trials, serum sickness was reported in 7 out of 285 (2%) patients. Three out of 7 (1%) patients had severe serum sickness.

Twenty-five events of acute systemic hypersensitivity reactions were reported in 16 patients. Acute systemic hypersensitivity reactions were managed by administration of adrenaline (44%; 11/25 episodes), corticosteroids (56%; 14/25), antihistamines (56%; 14/25), and/or oxygen (8%; 2/25 episodes). Four out of 16 (1%; 4/285) patients experienced a total of 5 episodes of acute systemic hypersensitivity reactions that were considered severe. Based on the immunogenicity profile, the underlying mechanism for acute systemic hypersensitivity reactions observed in clinical trials appears to be non-IgE mediated Type III (immune-complex mediated) hypersensitivity

Most of the events occurred in the first year of treatment and were dose-independent. Additional analysis did not identify any risk factors for the occurrence of ASHRs including age, gender, baseline and pre-event phenylalanine, cumulative dose and time from last dose to event onset.

The product information contains detailed warnings about the prevention and management of such events.

Due to the potential for an acute systemic hypersensitivity reaction, premedication prior to each dose is required during induction and titration (time prior to reaching blood phenylalanine levels less than 600 micromol/l while on a stable dose). Patients should be instructed to take premedication with an H1 antagonist, H2 antagonist, and antipyretic. During maintenance, premedication should be considered for subsequent injections based on patient tolerability to pegvaliase.

Prior to first dose of pegvaliase, the patient should be trained on the signs and symptoms of an acute systemic hypersensitivity reaction and to seek immediate medical care if a reaction occurs, and how to properly administer adrenaline injection device (auto injector or pre filled syringe/pen).

Initial administration(s) should be performed under supervision of a healthcare professional and patients should be closely observed for at least 60 minutes following of each of these initial injections. Prior to independent self -injection, a healthcare professional should train the patient and their observer, and assess patient competency on proper self-administration of this medicinal product.

For at least the first 6 months of treatment when the patient is self-injecting (i.e. when administration is not under healthcare professional supervision), an observer must be present during and for at least 60 minutes after each administration. An observer is present with the patient during and after pegvaliase administration, is able to recognise the signs and symptoms of an acute systemic hypersensitivity reaction, call for emergency medical support and administer adrenaline, if warranted. After 6 months of pegvaliase treatment, the need for an observer may be reconsidered.

An adrenaline injection device (auto-injector or pre-filled syringe/pen) should be prescribed to patients receiving this medicinal product. Patients should be instructed to carry adrenaline injection device with them at all times during pegvaliase treatment. Patients and the observer should be instructed to recognise the signs and symptoms of acute systemic hypersensitivity reactions, in the proper emergency use of the adrenaline injection device, and the requirement to seek immediate medical care. The risks associated with adrenaline use should also be considered when prescribing pegvaliase.

Prior to independent self-injection, a healthcare professional should:

- train the patient and assess patient competency on proper self-administration of this medicinal product.
- train the observer to recognise signs and symptoms of an acute systemic hypersensitivity reaction and to seek immediate medical care if a reaction occurs, and how to properly administer adrenaline injection device (auto-injector or pre-filled syringe/pen)

In addition, pegvaliase is contraindicated in patients who have experienced a severe systemic hypersensitivity reaction or recurrence of a mild to moderate acute systemic hypersensitivity reaction to

pegvaliase or another PEGylated medicinal product. For severe systemic hypersensitivity reactions or recurrence of a mild to moderate acute systemic hypersensitivity reaction, patients should seek immediate medical care and pegvaliase should be permanently discontinued. The prescribing physician should consider the risks and benefits of readministering the medicinal product following resolution of the first mild to moderate acute systemic hypersensitivity reaction. Upon readministration, the first dose must be administered with premedication under the supervision of a healthcare professional with the ability to manage acute systemic hypersensitivity reactions. Prescribing physician should continue or consider resuming use of premedication.

Acute systemic hypersensitivity reactions, and angioedema as a symptom of such reactions are included in the RMP as important identified risks. Educational material for Health Care Professionals and patients will also be distributed by the applicant. This additional risk minimisation activity is aimed at educating the HCPs for appropriate measures to be considered in order to reduce the incidence and severity of acute systemic hypersensitivity reactions and also educate the patients and trained observers to recognise signs of acute systemic hypersensitivity reactions so appropriate treatment can be initiated promptly, thus reducing the impact of the event.

The applicant will also conduct a prospective global observational study (study 165-501) to further characterize the risk of hypersensitivity events including the ASHRs. Notably patients enrolled in study 165-501 in case of an hypersensitivity reaction can be enrolled in substudy 165-503 for laboratory assessment. The primary objective of that study is to evaluate the immunologic and inflammatory responses (immunologic testing, inflammatory markers) associated with incidence of immune-mediated adverse reactions.

In the clinical trials, two patients on long term use of a PEGylated medroxyprogesterone acetate product experienced hypersensitivity reactions following single doses of pegvaliase. Verhoef and colleagues have proposed that immune-mediated side-effects of PEGylated products is based on the haptogenic properties of PEG, responsible for complement activation and the induction of anti-PEG antibodies (Verhoef 2014). Because antibodies bind to the PEG portion of pegvaliase, there may be potential for binding with other PEGylated therapeutics and increased hypersensitivity to other PEGylated injectables. The impact of anti-PEG antibodies on the clinical effects of other PEG containing medicinal products is unknown. For this reason, acute systemic hypersensitivity reactions in patients taking other concurrent injectables containing PEG is also included in the RMP as an important potential risk.

In the I/T/M Population, 125 (43.9%) patients met the definition of hypophenylalaninaemia (at least 2 consecutive blood phenylalanine levels <30 µmol/L). In 80% of the cases, blood phenylalanine levels dropped below 5 umol/L (lower limit of detection). The median time to onset from the first dose to the first hypophenylalaninaemia event was 393 days (range 51 to 1,405 days) and the median duration of hypophenylalaninaemia events was 161 days (range 35 to 1,408 days).

Measures were included in the protocol to prevent hypophenylalaninaemia (regular blood phenylalanine measurements, possibility for dose decrease and consultation of a dietician to increase protein intake). These measures have also been included in the SmPC in order to minimise this risk. Monitoring of blood phenylalanine level is recommended once a month. If a patient has a confirmed phenylalanine level below 30 micromol/l, dietary protein intake should be increased to appropriate levels, and then, if needed, the dosage of Palynziq should be reduced. (see section 4.2). In patients experiencing hypophenylalaninaemia despite appropriate levels of protein intake, dose reductions are expected to be most effective in managing hypophenylalaninaemia. Patients who develop hypophenylalaninaemia should be monitored every 2 weeks until blood phenylalanine level is within a clinically acceptable range. Based on animal studies, hypophenylalaninaemia in pregnant women with PKU treated with pegvaliase may be associated with adverse foetal outcomes. Blood phenylalanine levels should be monitored more frequently prior to and during pregnancy.

Since phenylalanine is an essential amino acid, there is a concern about possible long term possible adverse consequence and for this reason hypophenylalaninaemia has been included in the RMP as an important Identified risk and will be further characterised with a planned observational study to evaluate the long term safety of subcutaneous injections pegvaliase in adults with PKU (Study 16-501).

Persistent arthralgia (lasting at least 6 months) occurred in 19 (7%) patients with a total of 24 episodes. Persistent arthralgia is included in the RMP as an important identified risk as it may have a significant impact on the patient QOL. Additional pharmacovigilance activities (Studies PAL-003, 165-302, part 4 and 165-501) will further characterise the risk of persistent arthralgia with respect to number of reports, seriousness, outcome, and risk factors and whether experience in the post marketing setting is consistent with the information already known for this risk from clinical trial data.

93% of the patients experienced injection site reactions, and even though most of these reactions were not severe, it was acknowledged that with long term administration more severe reactions are possible. Severe injection site reactions may also have an impact on the patient QOL and are thus included in the RMP as an important identified risk which will be characterised in ongoing and planned studies. Appropriate warnings have been included in the product information to manage this risk.

Patients with pre-existing renal or hepatic impairment were not included in the clinical trials. Vacuoles were observed in chronic rat studies for pegvaliase, which may be consistent with the clearance of PEG over time. However, no associated kidney related toxicity was observed in the chronic toxicity rat studies with pegvaliase. Laboratory findings related also did not reveal any potential signals of significant renal toxicity. Patients with pre-existing renal or hepatic impairment are included in the RMP as missing information and information will be collected through the planned observational study.

Laboratory parameters were presented for the MD population. No safety concerns arose during the assessment of the laboratory findings.

No separate analysis was done on ethnicity since studies predominantly enrolled Caucasian Americans who will be representative of European ancestry, and therefore share a similar diversity of PKU gene mutations. Although the study population might not be representative for European patients with i.e. Asian ancestry, no safety concerns are anticipated.

Patients above 65 were not included in the studies and no conclusions can be drawn about the safety in elderly patients. The current data do not give rise to safety concerns in elderly patients. Use in the elderly is included in the RMP.

Immunogenicity assessment revealed that all patients develop ADA's. All patients developed sustained antibodies against the phenylalanine ammonia lyase (PAL) protein, and the majority of patients developed transient antibodies against polyethylene glycol (PEG). Anti-PAL responses peaked around 3-6 months and remained relatively stable through long term treatment (>1 year after pegvaliase initiation), indicative of a typical CD4-dependent antibody response induced against a foreign protein. Drug induced PEG IgM and PEG IgG responses peaked at 1-3 months after treatment initiation and then returned to baseline levels in most patients by 6-9 months of treatment suggestive of a CD4-independent antibody response typically induced against a repetitive, non-protein antigen. Neutralizing antibodies capable of inhibiting enzymatic activity of the PAL enzyme were detected in a majority of patients by approximately 6 months and were generally sustained once detected. Of the 13 patients who experienced acute systemic hypersensitivity reactions, all tested negative for drug-specific IgE at or near the time of an event. Observed increases in CIC levels in conjunction with C3/C4 complement consumption and lack of IgE detection, suggests that the predominant mechanism of injectionshypersensitivity.

In general, an association between ADA titers and frequency of hypersensitivity reactions and injection site reactions was observed for some antibody analytes, such that patients who had lower mean titers experienced fewer hypersensitivity reactions and injection site reactions. However, individual titers

overlapped between patients who experienced anaphylaxis events and those who did not; as a result, no specific antibody titers could be identified as predictive of an HAE or anaphylactic event. For the patients with high CIC formation, the decrease in complement C3/C4 was sustained with prolonged treatment. Additional analysis provided by the applicant showed suppressed complement was not associated with an increase in adverse events, with the exception of lymphadenopathy which was associated with decreased C3 levels. Complications of immune complex formation resulting in end-organ damage is included in the RMP as an important potential risk and will be further characterised in the ongoing studies PAL-003, part 4 of study 165-302and the planned immunogenicity/ inflammation lab study (165-503).

Assessment of paediatric data on clinical safety

A limited number of patients of 16-18 years were included in the studies. However, there seem to be no differences in the safety profile in the 16-18 years group and the 18-65 years group. The pegvaliase mechanism of action to correct the underlying metabolic deficiency of phenylalanine hydroxylase (PAH) gene is the same in both adults and adolescents (including 16 to <18 year olds). However, it is known that the innate and adaptive immune systems gradually mature over time. As the individual gets older, he or she develops an expanding repertoire comprising memory T and B cells triggered by previous infections and vaccinations, but also a naive-memory repertoire shaped by exposure to the microbiome, food antigens and inhaled antigens. For this reason, unpredictable immune-mediated response with off-label use in patients under 16 years of age have been included in the RMP as an important potential risk and will be addressed through the planned observational study (165-501).

2.6.2. Conclusions on the clinical safety

Despite the limited size of the safety database, due to the rarity PKU, the safety profile of pegvaliase has been adequately characterised. The main safety concern identified is the risk of acute systemic hypersensitivity reactions. To mitigate this risk, detailed warnings have been included in the product information to prevent and manage such events if they occur. Additional risk minimisation measures are also planned to further alert healthcare professionals and patients about this risk, and occurrence of such events will be closely monitored in an observational study.

The CHMP considers that the extensive risk minimisation measures that are in place is adequate to manage the risk of acute systemic hypersensitivity reactions and the other risks associated with the use of pegvaliase.

2.7. Risk Management Plan

Safety concerns

Summary of safety concerns	
Important identified risks	Acute systemic hypersensitivity reaction Angioedema Serum sickness Hypophenylalaninaemia Persistent arthralgia (≥ 6 months) Severe injection site reactions
Important potential risks	Complications of immune complex formation resulting in end-organ damage Foetal developmental toxicity Unpredictable immune-mediated response with off-label use in patients < 16 years

	Acute systemic hypersensitivity reactions in patients taking other concurrent injectables containing PEG
Missing information	Long term safety and tolerability Use in elderly (> 65 years) Use in patients with pre-existing renal impairment Use in patients with pre-existing hepatic impairment Use in breastfeeding women

Pharmacovigilance plan

Table 31. On-going and Planned Additional Pharmacovigilance Activities

Study Status	Summary of objectives	Safety concerns addressed	Milestones	Due Dates
Category 1 – Safety Studies imposed as condition of the marketing authorisation.				
Not applicable				
Category 2 – Safety studies which are specific obligations in the context of a marketing authorisation under exceptional circumstances or conditional marketing authorisation.				
Not applicable				
Category 3 – Safety Studies which are required by competent authority				
PAL-003 Ongoing	To assess Long-term safety	Acute systemic hypersensitivity reactions. Angioedema. Serum sickness. Hypophenylalaninaemia. Persistent arthralgia (≥ 6 months). Severe injection site reactions. Complications of immune complex formation resulting in end organ damage. Long-term safety and tolerability.	FPI: Interim CSR dates: LPO: Anticipated Date of Final CSR:	05 Jan 2010 Data cut-off 30 May 2017 30 Dec 2018 Q4, 2019
165-302 Part 4 Ongoing	To assess long-term safety	Acute systemic hypersensitivity reactions. Angioedema. Serum sickness. Hypophenylalaninaemia. Persistent arthralgia (≥ 6 months). Severe injection site reactions. Complications of immune complex formation resulting in end organ damage. Long term safety and tolerability.	FPI: Interim CSR dates LPO: Anticipated Date of Final CSR:	17 Oct 2013 28 Apr 2017 30 Sep 2018 Sep 2019

Study Status	Summary of objectives	Safety concerns addressed	Milestones	Due Dates
165-501 A prospective, global observational exposure study. Planned	**Primary Objective**: To quantify and further characterise important identified or potential risks associated with the use of pegvaliase in a real-world setting. Evaluated risks include, but are not limited to, severe immune-mediated ADRs (ie, acute systemic hypersensitivity reactions /anaphylaxis, angioedema, serum sickness, generalized skin reactions, arthralgia and hypersensitivity) and all ADRs of ASHR, angioedema and serum sickness. **Secondary Objectives**: • To estimate and further characterise potential major-organ function related, immune-mediated AEs and those resulting from PEG accumulation during pegvaliase treatment (eg, kidney function). • To quantify and further characterise adverse reactions of persistent arthralgia (≥ 6 months), as well as severe injection site reactions. • To quantify and characterise ASHRs and other immune mediated adverse reactions associated with concomitant use of pegvaliase and other PEG injectables. • To evaluate, quantify and characterise immune-mediated adverse reactions in subjects ≥ 65 years of age, patients < 16 years and in subjects with pre-existing hepatic or renal impairment. • To evaluate changes in blood Phe levels, including the quantification and further characterization of hypophenylalaninaemia (≤30 µmol/L), during pegvaliase treatment. • To evaluate changes in dietary protein intake during pegvaliase treatment. • To collect PKU disease history information that may include (but not limited to) date of diagnosis, severity	All important Identified and Potential risks will be assessed in this registry. Use in population not studied in the clinical trials: elderly > 65 years, hepatic impairment, renal impairment. The effectiveness of the risk minimisation activities for acute systemic hypersensitivity will also be measured.	Protocol submission FPI: Interim CSR dates: LPO: Anticipated Date of Final CSR:	Q2, 2019 Q4, 2019 Q2, 2022 and thereafter every 2 years Q4, 2029 Q2, 2030

Study Status	Summary of objectives	Safety concerns addressed	Milestones	Due Dates
	including PAH genotype, age of discontinuation of diet therapy (if applicable), history of other PKU therapies, and previous levels of Phe. **Tertiary objective (EU only):** • To evaluate patient-reported receipt of additional risk minimisation materials.			
165-503 An immunogenicity/ inflammation lab study (US) Planned	**Primary Objective**: Evaluate immunologic and inflammatory responses (immunologic testing, inflammatory markers) associated with incidence of immune-mediated adverse reactions. **Secondary Objectives**: • Evaluate immunologic and inflammatory responses (immunologic testing, inflammatory markers) and their effects on major-organ function (eg, kidney function). • Evaluate effect of immunologic responses on blood Phe.	Complications of immune complex formation resulting in end organ damage. Hypophenylalaninaemia.	Protocol submission FPI: Interim CSR dates: LPO: Anticipated Date of Final CSR:	Q2, 2019 Q4, 2019 Q2, 2022 and thereafter every 2 years Q4, 2029 Q2, 2030
165-504 A prospective global pregnancy observational safety surveillance study Planned	**Primary objective** To estimate the frequency of selected pregnancy outcomes (eg, spontaneous abortion, stillbirth, and preterm delivery) among women with PKU exposed to pegvaliase during pregnancy and infant outcomes among their offspring exposed to pegvaliase during pregnancy or during breastfeeding. **Secondary Objective**: • To examine differences in the frequency of selected pregnancy outcomes and foetal outcomes among women with PKU exposed to pegvaliase during pregnancy and their offspring by maternal blood Phe levels.	Foetal developmental toxicity. Use in breastfeeding	Protocol submission Interim CSR dates: Anticipated Date of Final CSR:	Q2, 2019 Q2, 2022 and thereafter every 2 years Q2, 2030
BMN165-18-080 Peri-/postnatal development study in rats Planned	To detect potential adverse effects of rAvPAL-PEG on Crl:CD(SD) female rats and development of their offspring consequent to exposure of the female from implantation through lactation and weaning.	Foetal developmental toxicity.	Protocol Submission Anticipated Date of Final CSR:	Q1, 2019 Q4, 2020

Study Status	Summary of objectives	Safety concerns addressed	Milestones	Due Dates
Foetal development study in rabbits Planned	To compare foetal malformations in pregnant rabbits caused by dietary restriction of phenylalanine to foetal malformations in pregnant rabbits treated with pegvaliase.	Foetal developmental toxicity	Protocol submission	Q1, 2020
Immune tolerance induction (ITI) regimen study Planned	To evaluate the ability of the ITI regimen (given prior to or concurrently with pegvaliase) to suppress immune responses, to reduce the risks of immune-mediated adverse reactions, and to enable improved therapeutic responses in adult patients with PKU treated with pegvaliase.	Immune mediated adverse reactions	Protocol submission	Within 6 months of EC Decision

Risk minimisation measures

Table 32. Summary Table of Risk Minimisation Measures by Safety Concern

Safety Concern	Risk minimisation measures
Acute systemic hypersensitivity reactions (Important Identified Risk)	Routine risk minimisation measures: SmPC Sections 4.2, 4.3, 4.4, 4.7, 4.8. PL Sections 2, 3, 4, 7. Additional risk minimisation measures: Educational materials for HCPs. Educational materials for Patients and Trained Observers. Patient alert card.
Angioedema (Important Identified Risk)	Routine risk minimisation measures: SmPC Section: 4.4, 4.8. PL Section: 2, 4. Additional risk minimisation measures: As angioedema may be a symptom of acute systemic hypersensitivity reactions, the same additional risk minimisation measures will apply. Educational materials for HCPs. Educational materials for Patients and Trained Observers. Patient alert card.
Serum sickness (Important Identified Risk)	Routine risk minimisation measures: SmPC Section: 4.4, 4.8. PL: Section 4. Additional risk minimisation measures: None.
Hypophenylalaninaemia (Important Identified Risk)	Routine risk communication: SmPC Section: 4.2, 4.4, 4.6. PL: Section 2.

Safety Concern	Risk minimisation measures
	Additional risk minimisation measures: None.
Persistent arthralgia (≥ 6 months) (Important Identified Risk)	Routine risk communication: SmPC Section: 4.8. PL: Section 4. Additional risk minimisation measures: None.
Severe injection site reactions (Important Identified Risk)	Routine risk communication: SmPC Section: 4.2, 4.8. PL: Section 4. Additional risk minimisation measures: None.
Complications of immune complex formation leading to end organ damage (Important Potential Risk)	Routine risk minimisation measures: None. Additional risk minimisation measures: None.
Foetal developmental toxicity (Important Potential Risk)	Routine risk communication: SmPC Section: 4.4, 4.6. PL: Section 2. Additional risk minimisation measures: None.
Unpredictable immune-mediated response with off-label use in patients < 16 years (Important Potential Risk)	Routine risk communication: SmPC Section: 4.2. PL: Section 2. Additional risk minimisation measures: None.
Acute systemic hypersensitivity reactions in patients taking other concurrent injectables containing PEG (Important Potential Risk)	Routine risk communication: SmPC Section: 4.4. PL: Section 2. Additional risk minimisation measures: None.
Long term safety and tolerability (Missing Information)	Routine risk minimisation measures: None. Additional risk minimisation measures: None.
Use in elderly > 65 years	Routine risk minimisation measures: None

Safety Concern	Risk minimisation measures
(Missing Information)	Additional risk minimisation measures: None.
Use in patients with pre-existing hepatic impairment (Missing Information)	Routine risk minimisation measures: SmPC Section 5.2. Additional risk minimisation measures: None.
Use in patients with pre-existing renal impairment (Missing Information)	Routine risk minimisation measures: SmPC Section 5.2. Additional risk minimisation measures: None.
Use in breastfeeding women (Missing Information)	Routine risk minimisation measures: SmPC Section 4.6. PL Section: 2. Additional risk minimisation measures: None.

Conclusion

The CHMP and PRAC considered that the risk management plan version 0.5 is acceptable.

2.8. Pharmacovigilance

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.

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 request alignment of the PSUR cycle with the international birth date (IBD). The IBD is 24.05.2018. The new EURD list entry will therefore use the IBD to determine the forthcoming Data Lock Points.

2.9. New Active Substance

The applicant declared that pegvaliase has not been previously authorised in a medicinal product in the European Union.

The CHMP, based on the available data, considers pegvaliase to be a new active substance as it is not a constituent of a medicinal product previously authorised within the Union.

2.10. Product information

2.10.1. 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.

2.10.1. Additional monitoring

Pursuant to Article 23(1) of Regulation No (EU) 726/2004, Palynziq (pegvaliase) 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

Palynziq is indicated for the treatment of patients with phenylketonuria (PKU) 16 years and older who have inadequate blood phenylalanine control (blood phenylalanine levels greater than 600 µmol/L) despite prior management with available treatment options.

PKU is an inherited, autosomal recessive, non-lethal disease characterized by a deficiency in the liver enzyme, phenylalanine hydroxylase (PAH). PAH catalyses the conversion of the essential amino acid phenylalanine to tyrosine, and this enzymatic activity is facilitated by tetrahydrobiopterin (BH4). PAH deficiency results in abnormally elevated concentrations of phenylalanine, which is toxic to the brain. PKU in children and adults can be controlled by the reduction of blood phenylalanine levels. The recommended blood phenylalanine level in the EU guideline for the treatment of PKU is 120 -600 µmol/L for both children over 12 years and adults.

There is also a high incidence of neurologic and psychiatric disorders (including intellectual disability, anxiety, depression, and neurocognitive dysfunction) in the adult PKU patient population. In addition, these patients have impaired working memory, inhibitory control and social-cognitive functioning compared to healthy controls.

3.1.1. Available therapies and unmet medical need

Current management of PKU is based on restriction of dietary protein to reduce phenylalanine intake to levels that will allow maintenance of target phenylalanine levels and ingestion of phenylalanine-free protein supplements to maintain daily protein requirements. Patients may tolerate only a small fraction of the usual daily intake of protein and must meet >80% of daily protein requirements using phenylalanine-free protein supplements.

Maintenance of this degree of protein restriction is impractical in daily life, also considering that the phenylalanine-free protein supplement is known to be unpalatable. Poor compliance with diet management is a well-known occurrence in adults with PKU.

If the patient cannot adhere to diet, sapropterin can be administered concomitantly. However, a large proportion (around 60% in Northern Europe and around 40% in Southern Europe) of (adult) PKU patients will not benefit from sapropterin treatment because sapropterin is effective in a limited number of patients only that have residual PAH activity. Sapropterin is available as a tablet or powder for oral solution and is usually taken once a day with food.

There is currently an unmet medical need in adult patients with PKU who have uncontrolled blood phenylalanine levels (i.e. who failed to achieve guideline recommended blood phenylalanine control) with the combination of the currently available treatment sapropterin and phenylalanine-restricted diet.

3.1.2. Main clinical studies

Two phase III studies (study 165-301 and 165-302) were submitted of which study 165-302 is considered as the main study, consisting of four parts. Part 1 was a 13 weeks run-in phase to reach and maintain a stable pegvaliase dose for those patients that were transitioned from the phase II studies 165-205 and PAL-003 or to further maintain the patients transitioned from study 165-301 on a stable dose; part 2 was a 8 weeks double blind placebo-controlled randomized withdrawal part; part 3 consisted of a six week PK period; and part 4 was an open label long term follow-up period up to 212 weeks including a titration based on phenylalanine levels randomized study in the clinical program, part 4 of this study investigates long term efficacy of pegvaliase.

3.2. Favourable effects

Titration phase

In general 95% of the patients reached their randomized dose in less than 23.9 weeks. A higher percentage of patients in the 40 mg/day randomized dose group compared to the 20 mg/day randomized dose group achieved blood phenylalanine reductions to <600 µmol/L; 46.9% *versus* 34.4%.

Maintenance phase

93.4% of the patients received their randomized dose level as their final dose. A further reduction of blood phenylalanine levels was observed after dose increases (maximal allowed dose was 60 mg/day); median change from baseline part 4 up to week 41 was -1109.0 µmol/l in the <20 mg/day (n=38), -1126.0 µmol/l in the 20 -<40 mg/day (n=112), -761 µmol/l in the 40-<60 mg/day (n=181) and -516.0 µmol/l in the ≥60 mg/day group. Overall, 65.3% of the patients reached a phenylalanine level ≤600 µmol/L from baseline study 165-301 to week 41 in study 165-302 part 4. Thirty-one patients who were in the 40 mg/day group and did not achieve blood phenylalanine concentration <600 µmol/L, when titrated to 60 mg/day 24/31 patients reached the target threshold.

Under continued pegvaliase treatment patients were capable in increasing their protein intake from intact food.

Median changes (expressed in points) in ADHD Inattention Score mean from baseline part 4 to week 41 for the difference dosing categories were -5.0 in the <20 mg/day (n=42), -3.0 in the ≥20 -<40 mg/day (n=130), -4.0 in the ≥40-<60 mg/day (n=185) and -5.5 in the ≥60 mg/day (n=69) group.

After 24 months of pegvaliase treatment these scores were -6.5 in the <20 mg/day (n=19), -6.0 in the ≥20 -<40 mg/day (n=23), -6.3 in the ≥40-<60 mg/day (n=84) and -4.2 in the ≥60 mg/day (n=34) group when compared to baseline.

Additional analyses confirmed that under continued treatment the ADHD Inattention Score, PKU POMS TMD and POMS TMD (Self-Rated) exceeded the minimal important clinical difference. A greater magnitude of reduction was observed with long-term treatment in patients who had a baseline ADHD Inattention Subscale >9 at baseline.

3.3. Uncertainties and limitations about favourable effects

Of the 261 PKU patients initially included in study 165-301, 190 patients enrolled in part 4 of study 165-302. 27% of the patients discontinued due to AEs or other reasons (e.g. lost to follow-up) prior to enrolment in part 4. This discontinuation rate impacts the responder analysis of part 4 of study 165-302.

The poolability testing of the placebo groups (20 mg/day and 40 mg/day) indicated a significant difference ($p = 0.0424$), suggesting that the placebo groups should not be pooled. However, as this pooling was pre-specified due to lower enrolment rates and individual comparisons did not show different results, pooled analyses were accepted but should be considered cautiously.

In the open-label part 4 of study 165-302 data on neurocognitive and neuropsychiatric symptoms showed some, non-statistically significant improvement and too small to be clinically relevant in patients still on treatment, further the open-label design limits the robustness of the conclusion drawn.

An increased intake of natural foods and phenylalanine, is only reported for patients still on treatment in the open-label phase of the study. Therefore, data should be interpreted cautiously.

3.4. Unfavourable effects

Pegvaliase is administered in an induction/titration/maintenance regimen to enhance tolerability. The relevant population for safety assessment is the I/T/M population consisting of 285 patients.

Drug related AEs occurred in 100% of patients. The most common drug-related AEs among patients were injection site reactions (93.3%), arthralgia (84.6%), hypersensitivity reactions (74.7%), headache (54.7% rash (38.9%), nausea (35.1%), pruritus (31.9%) and urticaria (30.5%).These adverse events are considered related to the hypersensitivity reactions observed in all patients. The incidence of HAEs in the placebo group was 14.3% versus 79.6% in the <20 mg/day group (highest incidence) and 41.7% in the ≥60 mg/day group (lowest incidence).

A number of patients (5.6%) experienced acute systemic hypersensitivity reactions (ASHRs) which were time and dose-independent.

Angioedema, not as part of an acute hypersensitivity reaction, was experienced in 21 patients (7.4%) in the I/T/M population. All events were grade 1 and 2 events. Serum sickness was reported in 7 patients (2.5%), of which 3 patients had grade 3 events. For 2 patients, the drug was withdrawn due to serum sickness (listed as SAEs), the remaining 5 events were managed with drug disruption, drug reduction or concomitant medication. All events of serum sickness resolved without sequelae.

All patients developed ADAs against either pegvaliase (anti-pal) or PEG (anti-peg). In 70-80% of the patients, neutralizing antibodies (Nab, capable of inhibiting the enzymatic activity of pegvaliase) were detected. In general, hypersensitivity AEs and specifically acute systemic hypersensitivity reactions were most commonly seen in patients which had the highest antibody titres/CIC levels (29.6% in quartile 3+4 versus 12.3 % in quartile 1+2), but individual titres were not predictive for the drug tolerability.

Hypophenylalaninaemia, defined as when a patient who had at least 2 consecutive blood phenylalanine levels <30 µmol/L, occurred in 125 (43.9%) of patients in the I/T/M Population. The median duration was 162 days. In 80% of the patients experiencing hypophenylalaninaemia, blood phenylalanine levels dropped below 5 µmol/L (lower limit of detection of the assay).

3.5. Uncertainties and limitations about unfavourable effects

No risk factors could be identified for the occurrence of ASHRs. In 11 of the 25 events (44%) epinephrine was administered. Due to the occurrence of acute systemic hypersensitivity events early in the clinical study program, risk minimization measures were instated including the use of pre-medications during titration, a prolonged titration phase, the need to carry an epi-pen and the presence of a trained observer. This risk will be further characterised in the ongoing PAL-003 clinical study and the planned observational pegvaliase exposure study (165-501).

The risks associated with hypophenylalaninaemia are poorly understood, hypophenylalaninaemia is included in the safety specifications and will be addressed in the proposed observational pegvaliase exposure study (study 165-501).

3.6. Effects Table

Table 33. Effects Table for pegvaliase for the treatment of patients with phenylketonuria aged 16 years and older who have inadequate blood phenylalanine control despite prior management with available treatment options (data cut-off: 5th February 2018).

Effect	Short description	Unit	Treatment	Control	Uncertainties / Strength of evidence	References
Favourable Effects						
Change in blood Phe	Change from baseline LS mean (95% CI)	µmol/L	Pooled active - 20 mg/day vs placebo: -923.3 (-1135.04 to -711.46) Pooled active - 40 mg/day vs placebo: -638.3 (-858.97 to -417.57)		Data confirm the PD effect of pegvaliase (p<0.0001). Of the initial PKU patients included in study 165-301 about 27% of the patients discontinued prior to enrolment in part 4 of study 165-302	*Study 165-302 part 2*
Change in ADHD Inattention score	Mean (SD) change from baseline to month 24	points	<20 mg/day: -6.0 (5.4) ≥20 - <40 mg/day : -6.0 (5.4) ≥40- <60 mg/day : -6.3 (6.0) ≥60 mg/day : -4.2 (6.1)		Data is based on all 261 patients who started on pegvaliase in study 165-301. Data pertains to open label data only. Similar results were observed for the PKU-POMS assessment.	*Study 165-301* *Study 165-302 part 4*
Change in intake of natural proteins	median (min, max)	%	RDT population: 79.3 (-42, 727) non-RDT population: 29.7 (-77, 358)		Consistent results throughout clinical studies	*Study 165-302 part 4*
Unfavourable Effects						
Acute systemic hyper-sensitivity	NIAID/FAAN clinical diagnostic criteria for anaphylaxis,	n (%)	13/285 patients (4.5)	N/A	25 events in total, majority occurring in the first year of treatment	ITM population
Hypophenyl-aninaemia	At least 2 consecutive blood Phe levels <30 µmol/L	n (%)	125/285 (43.9)	N/A	Clinical consequence of events unclear	
Immuno-genicity	Neutralizing antibodies at week 36	n (%)			Data indicates that from baseline to week 4 (desensitization phase) patients may develop Nabs	

Abbreviations: *Phe: Phenylalanine, LS: Least Squares, CI: Confidence interval, SD: Standard Deviation, NIAID/FAAN: National Institute of Allergy and Infectious Disease and the Food Allergy and Anaphylaxis Network, N/A:*

Not applicable

I/T/M: Induction, Titration, Maintenance

3.7. Benefit-risk assessment and discussion

3.7.1. Importance of favourable and unfavourable effects

Under continued pegvaliase treatment, the majority of treated patients reached the target of phenylalanine level at week 41 (part 4 of study 165-302). In addition, in the randomised withdrawal phase (part 2) of study 165-302, it was demonstrated that in patients who were withdrawn from pegvaliase (e.g. randomised to placebo treatment) blood phenylalanine levels increased. When treatment was re-introduced (in part 3) blood phenylalanine levels decreased again under continued pegvaliase treatment up to 41 week in the long-term open label part of the study part (part 4). These findings clearly demonstrate, together with the observed reduction of blood phenylalanine in the other studies, the pharmacodynamic properties of pegvaliase. This effect is considered, clinically meaningful and beneficial.

Data indicates that under continued long-term treatment the neuropsychiatric/ neurocognitive symptoms further improve.

Patients with blood phenylalanine levels ≤600 µmol/L could increase their intake of dietary phenylalanine and proteins from intact food. Protein intake seemed to normalise towards protein intake for healthy subjects. Given the widely acknowledged unpalatability of medical grade food this is considered an additional benefit for the patient.

No differences in efficacy and safety were observed in the 12 paediatric patients (16 to <18 years old) included in the clinical program compared to the patients >18 years old.

In the clinical program patients were titrated based on tolerability to a fixed dose of pegvaliase (e.g., 20 mg/day or 40 mg/day) and the study design only facilitated for further optimisation of the dose in the long term follow-up phases of the clinical program (patients could be titrated up to 60 mg/day or titrated down as far as to 5 mg/day to reach an optimal blood phenylalanine concentration). In this context, it is anticipated that in clinical practice the patients with PKU are titrated to the most optimal dose to achieve blood phenylalanine levels between 120 and ≤600 µmol/L taking in account the patients' tolerability to pegvaliase. The posology scheme is in line with anticipated clinical practice.

Acute and clinically relevant systemic hypersensitivity reactions were observed in 5.6% of treated patients. These reactions were predominantly observed in the first year of treatment. Risk factors for the occurrence of ASHRs could not be identified. In a substantial proportion of patients (44%) presenting with ASHR treatment with epinephrine was needed. In the clinical study program risk minimization measures were instated to manage ASHRs. These measures were able to decrease the incidence and severity of hypersensitivity reactions and the associated discontinuations. These measures are reflected in the SmPC of pegvaliase, and additional educational material for prescribers and patients are intended to further minimise and prevent the occurrence of these events in clinical practice.

Other risks associated with pegvaliase use, such as hypophenylaninaemia and occurrence of neutralising antibodies are considered to be adequately managed by routine risk minimisation measures. Further information on the incidence, severity and clinical consequences of such adverse reactions are expected through planned and ongoing studies which are described in the RMP.

3.7.2. Balance of benefits and risks

The clinical efficacy of pegvaliase in terms of a pharmacodynamic effect of pegvaliase has been convincingly demonstrated. For the overall benefit-risk assessment the effect of lowering blood

phenylalanine levels on dietary protein intake and neurocognitive/neuropsychiatric symptom scores is also important.

The majority of the AE experienced were mild-moderate hypersensitivity adverse events. A part of the patients experiences serious adverse events of which the majority were serious acute systemic hypersensitivity events. AEs led to a substantial treatment discontinuation rate, especially in the early treatment phase.

These events are expected to be managed by stringent risk minimization measures, including mandatory premedication, the presence of a trained observer and a more flexible titration phase, which in the clinical trials were shown to be effective in decreasing drug discontinuations and ASHRs/HAEs.

However, in general the AEs were tolerated, manageable and resolved without sequelae.

The long-term effect of treatment on safety in PKU patients will be further evaluated in the planned observational study.

3.8. Conclusions

The overall B/R of Palynziq is positive.

4. Recommendations

Similarity with authorised orphan medicinal products

The CHMP by consensus is of the opinion that Palyzinq is not similar to Kuvan within the meaning of Article 3 of Commission Regulation (EC) No. 847/2000. See appendix 1.

Outcome

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considers by consensus that the benefit-risk balance of Palynziq is favourable in the following indication:

Treatment of patients with phenylketonuria (PKU) aged 16 years and older who have inadequate blood phenylalanine control (blood phenylalanine levels greater than 600 micromol/l) despite prior management with available treatment options.

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 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.

Additional risk minimisation measures

Prior to launch of Palynziq in each Member State, the Marketing Authorisation Holder (MAH) must agree about the content and format of the educational programme, including communication media, distribution modalities, and any other aspects of the programme, with the National Competent Authority.

The MAH shall ensure that in each Member State where Palynziq is marketed, all healthcare professionals and patients, carers and observers who are expected to prescribe, use or oversee the administration of Palynziq have access to/are provided with the following educational package:

- Physician educational material
- Patient information pack

- **The physician educational material** should contain:
  - The Summary of Product Characteristics
  - Guide for healthcare professionals
- **The Guide for healthcare professionals** shall contain the following key elements:
  - Information on the risk of acute systemic hypersensitivity reactions and details of the risk minimisation measures necessary to minimise this risk (i.e. premedication, trained observer, prescription of adrenaline injection device).
  - Management of acute systemic hypersensitivity reactions and information on retreatment
  - Key messages that must be conveyed and elements that must be addressed prior to self-injection by the patient, in particular:
    - training of patients to recognise the signs and symptoms of acute systemic hypersensitivity reactions and the action to be taken if such a reaction occurs
    - prescription of adrenaline injection device and training on its use
    - premedication requirements
    - provision of appropriate instruction on self-administration of pegvaliase
    - assessment of competency in self-injection by patient

- requirement for a trained observer for at least the first 6 months of treatment
- training of the observer to recognise the signs and symptoms of acute systemic hypersensitivity reactions, to seek immediate medical care if a reaction occurs, and how to properly administer adrenaline injection device
- provision of the guide for patients and trained observers and patient alert card

- Information about the observational study to evaluate long term safety and the importance of contributing to such study where applicable

- **The patient information pack** should contain:
  - The patient information leaflet
  - The guide for patients and trained observers
  - The patient alert card

  The guide for patients and trained observers shall contain the following key messages:
  - Description of the signs and symptoms of severe allergic reactions
  - Information on the action to be taken by the patient and/or trained observer in the event of the occurrence of a severe allergic reaction
  - Description of the risk minimisation measures necessary to minimise the risk of severe allergic reactions, in particular:
    - Premedication requirements
    - Requirement to carry adrenaline injection device at all times
    - Requirement for trained observer for at least the first 6 months of treatment
  - The need to contact the prescriber in the event of a severe allergic reaction prior to continuing treatment
  - The importance of carrying the patient alert card

  The patient alert card shall contain the following key messages:
  - A warning message for HCPs treating the patient at any time, that the patient is using Palynziq and severe allergic reactions have been associated with this product
  - Signs or symptoms of the severe allergic reactions and action to be taken in the event of such a reaction
  - The importance of carrying an adrenaline injection device and the patient alert card at all times

Emergency contact details for the patient and contact details of the prescriber

New Active Substance Status

Based on the CHMP review of the available data, the CHMP considers that pegvaliase is a new active substance as it is not a constituent of a medicinal product previously authorised within the European Union.