12 October 2023
EMA/485896/2023
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Loargys

International non-proprietary name: pegzilarginase

Procedure No. EMEA/H/C/005484/0000

Note

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

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List of abbreviations

Abbreviation-	Definition
2MWT	Two-Minute Walk Test
6MWT	Six-Minute Walk Test
ADA	Antidrug Antibody
AE	Adverse Event
AESI	Adverse Event Of Special Interest
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
ARG1	Arginase 1
ARG1-D	Arginase 1 Deficiency
AST	Aspartate Aminotransferase
AUC_{0-168}	Area Under The Serum Concentration-Time Curve From 0 To 168 Hours Postdose
BL	Baseline
CCIT	Container Closure Integrity Testing
CCS	Container Closure System
CHMP	Committee for Medicinal Products for Human Use
CI	Confidence Interval
C_{max}	Maximum Serum Concentration
CQA	Critical Quality Attribute
CSR	Clinical Study Report
DB	Double Blind
DNA	Deoxyribonucleic Acid
DOE	Design Of Experiment
DSI	Drug (Active) Substance Intermediate
E. coli	Escherichia Coli
EAA	Essential Amino Acid
EBD	EU Birth Date
EC	European Commission
E-IMD	European Registry And Network For Intoxication Type Metabolic Diseases
EMA	European Medicines Agency
EoPC	End Of Production Cell
EURD	European Union Reference Date
FAS	Full Analysis Set
FDBU	Fujifilm Diosynth Site
FMEA	Failure Modes And Effect Analysis
GC	Guanidino Compound
GCP	Good Clinical Practice
GD	Gestation Day
GLP	Good Laboratory Practice
GLS	Geometric Least Squares
GMFCS	Gross Motor Function Classification System

GMFM	Gross Motor Function Measure
GMFM-D	Gross Motor Function Measure Item D (Standing)
GMFM-E	Gross Motor Function Measure Item E (Walking, Running, And Jumping)
GMP	Good Manufacturing Practice
HA	Hyperammonaemia
hArg1	Human Arginase 1
HPLC	High Performance Liquid Chromatography
IBD	International Birth Date
ICH	International Council For Harmonization Of Technical Requirements For Pharmaceuticals For Human Use
icIEF	Imaged Capillary Isoelectric Focusing
IP	Intraperitoneal
IPC	In-Process Control
ISR	Incurred sample reanalyses
IV	Intravenous(Ly)
kDa	Kilodalton
LFT	Liver Function Test
LLOQ	Lower Limit Of Quantification
LS	Least Squares
LTE	Long-Term Extension
max	Maximum
MCB	Master Cell Bank
MCID	Minimum Clinically Important Difference
MedDRA	Medical Dictionary For Regulatory Activities
min	Minimum
MMRM	Mixed Model Repeated Measures
mPEG	Methoxy Polyethylene Glycol
MTD	Maximum Tolerated Dose
NCA	Non-Compartmental Analysis
NOAEL	no-observed-adverse-effect level
nor-NOHA	Nω-hydroxy-nor-arginine
OFAT	One Factor A Time
p[Arg]	Plasma Arginine Concentration
PACMP	Post-Approval Change Management Protocol
PASS	Post Authorisation Safety Study
PAES	Post Authorisation Efficacy Study
PD	Pharmacodynamic(S)
PDE	Permitted Daily Exposure
PEG	Polyethylene Glycol
Ph. Eur.	European Pharmacopoeia
PK	Pharmacokinetic(S)
PND	Post-natal day
PP	Per-Protocol
PPQ	Process Performance Qualification

PRS	Primary Reference Standard
PSUR	Periodic Safety Update Report
PT	Preferred Term
QA	Quality Attributes
QbD	Quality By Design
QC	Quality Control
QW	Once Weekly
rhARG1	Recombinant Human Arginase 1
RMP	Risk Management Plan
SAE	Serious Adverse Event
SC	Subcutaneous(Ly)
SD	Standard Deviation
SmPC	Summary Of Product Characteristics
SOB	Specific Obligation
SOC	System Organ Class
$t_{1/2}$	Half-Life
TE	Treatment-Emergent
TEAE	Treatment-Emergent Adverse Event
TK	Toxicokinetic
TSE	Transmissible Spongiform Encephalopathies
UCD	Urea Cycle Disorder
ULN	Upper Limit Of Normal
USP	United States Pharmacopoeia
WCB	Working Cell Bank

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Immedica Pharma AB submitted on 26 July 2022 an application for marketing authorisation to the European Medicines Agency (EMA) for Loargys, 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 17 October 2019.

Loargys was designated as an orphan medicinal product EU/3/16/1701 on 14 July 2016 in the following condition: treatment of hyperargininaemia.

Following the CHMP positive opinion on this marketing authorisation, the Committee for Orphan Medicinal Products (COMP) reviewed the designation of Loargys 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: https://www.ema.europa.eu/en/medicines/human/Loargys

The applicant applied for the following indication: treatment of arginase 1 deficiency (ARG1-D), also known as hyperargininaemia, in adults, adolescents and children aged 2 years and older.

1.2. Legal basis and dossier content

The legal basis for this application refers to:

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

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

1.3. Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision P/0252/2020 on the agreement of a paediatric investigation plan (PIP).

At the time of submission of the application, the PIP P/0252/2020 was not yet completed as some measures were deferred.

1.4. Information relating to orphan market exclusivity

1.4.1. Similarity

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

1.5. Applicant's requests for consideration

1.5.1. Marketing authorisation under exceptional circumstances

The applicant requested consideration of its application for a marketing authorisation under exceptional circumstances in accordance with Article 14(8) of the above-mentioned Regulation.

1.5.2. New active substance status

The applicant requested the active substance pegzilarginase 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.

1.6. Protocol assistance

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

Date	**Reference**	**SAWP co-ordinators**
18 October 2018	EMEA/H/SA/3838/1/2018/PA/SME/III	Dr Hans Ovelgönne and Prof. Brigitte Blöchl-Daum
28 March 2019	EMEA/H/SA/3838/1/FU/1/2019/PA/SME/II	Dr Karin Janssen van Doorn, Dr Hans Ovelgönne

The protocol assistance pertained to the following non-clinical and clinical aspects:

- Agreement on the applicant's planning of non-clinical testing, including reproductive toxicology studies
- Agreement on the proposed single pivotal phase 3 trial design, including the primary endpoint and selected clinical outcome measures for the secondary endpoints
- The clinical data package to support the marketing authorisation application

1.7. Steps taken for the assessment of the product

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

Rapporteur: Peter Mol Co-Rapporteur: Ewa Balkowiec Iskra

The application was received by the EMA on	26 July 2022
The procedure started on	18 August 2022
The CHMP Rapporteur's first Assessment Report was circulated to all CHMP and PRAC members on	7 November 2022
The CHMP Co-Rapporteur's first Assessment was circulated to all CHMP and PRAC members on	14 November 2022

The PRAC Rapporteur's first Assessment Report was circulated to all PRAC and CHMP members on	22 November 2022
The CHMP agreed on the consolidated List of Questions to be sent to the applicant during the meeting on	15 December 2022
The applicant submitted the responses to the CHMP consolidated List of Questions on	15 May 2023
The following GCP inspection was requested by the CHMP and their outcome taken into consideration as part of the Safety and Efficacy assessment of the product:	
– A GCP inspection at the sponsor site and two investigator sites in the United Kingdom and United States between 12 Dec 2022 – 20 Jan 2023. The outcome of the inspection carried out was issued on	17 March 2023
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Questions to all CHMP and PRAC members on	26 June 2023
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	06 July 2023
The CHMP agreed on a list of outstanding issues in writing and/or in an oral explanation to be sent to the applicant on	20 July 2023
The applicant submitted the responses to the CHMP List of Outstanding Issues on	08 September 2023
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP and PRAC members on	27 September 2023
The outstanding issues were addressed by the applicant during an oral explanation before the CHMP during the meeting on	N/A
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 Loargys on	12 October 2023
The CHMP adopted a report on similarity of Loargys with Ravicti on	12 October 2023
Furthermore, the CHMP adopted a report on New Active Substance (NAS) status of the active substance contained in the medicinal product on	12 October 2023

2. Scientific discussion

2.1. Problem statement

2.1.1. Disease or condition

ARG1-D (arginase-1 deficiency) is a rare, debilitating, progressive, inherited, neurodegenerative, metabolic disease associated with increased arginine and its toxic metabolites. ARG1-D is associated with significant reductions in quality of life, increased morbidity (lower-limb spasticity), and premature mortality. It is an autosomal recessive disease caused by a deficiency in the ARG1 enzyme, which is an essential step in the urea cycle. ARG1 is mainly expressed in the liver and red blood cells. There are at least 60 potentially disease-causing variations in ARG1 gene reported, with the majority being missense /nonsense mutations and small deletions.

2.1.2. Epidemiology

ARG1-D is one of the least common of the urea cycle disorders (UCDs). ARG1-D is estimated to account for approximately 3.5% of all UCD cases. Findings of a genetic analysis based on mathematical modelling estimated a similar global birth prevalence for ARG1-D of 2.8 cases per million live births (1:357,000 live births), and a population prevalence of 1.4 cases per million people (approximately 1:726,000 people). An estimate based on newborn screening of other UCDs suggested an incidence of 1:950,000, which, with a 2019 EU birthrate of 4.17 million (Eurostat), correlates to 4-5 newborns in the European Union (EU) annually. The applicant is aware of 70-80 patients diagnosed with ARG1-D in the EU.

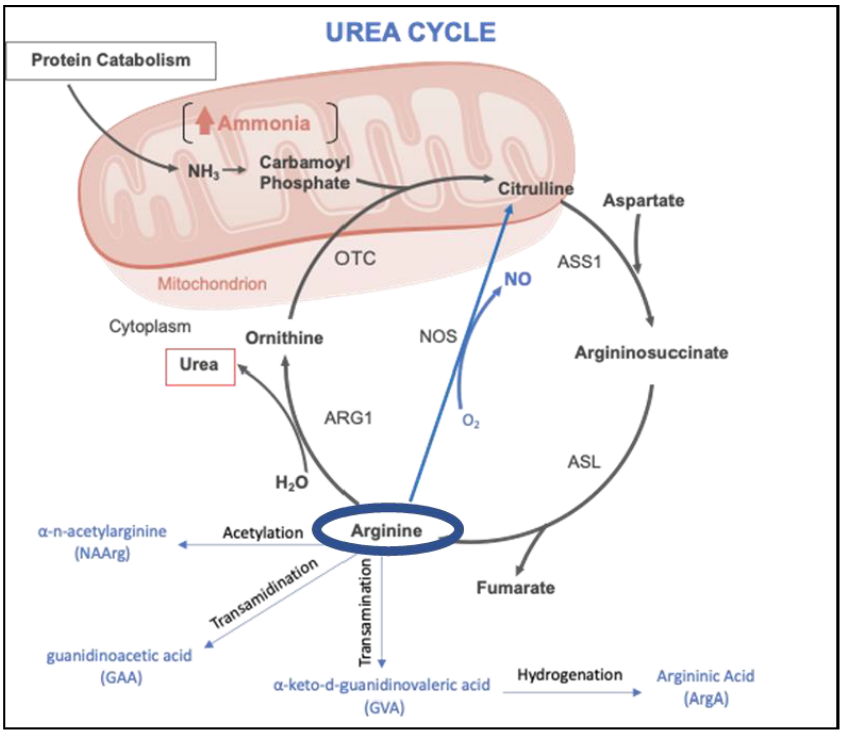
2.1.3. Biologic features, aetiology and pathogenesis

The role of the urea cycle is to detoxify waste nitrogen by producing urea from ammonia. The urea cycle consists of five consecutive enzymatic reactions distributed between the mitochondria and the cytosol, as well as two transporters mediating the transport of urea cycle intermediates between mitochondria and cytosol (

Figure 1). The final enzyme reaction within the urea cycle is by ARG1 hydrolysis of arginine to ornithine and urea. Urea can thereby be excreted by the kidneys, whereas ornithine is returned to the mitochondria to continue the cycle.

Arginase-1 is mainly expressed in the liver, the main organ of protein metabolism, while arginase-2 is mainly expressed in the CNS. Circulating plasma arginine is widely distributed into the organs, including the brain, via cationic amino acid transport (CAT) systems (of any subtype). Accumulation of arginine in the CSF has been found in patients and animal models with ARG1-D. There was a strong and direct correlation between arginine levels in plasma and CSF.

Figure 1 Metabolic effects of arginase 1 deficiency



adapted from Blair NF, 2014.

All patients with ARG1-D have an impaired ARG1 enzyme, with a decreased or non-existent activity that leads to the accumulation of arginine and its neurotoxic metabolites, guanidino compounds (GCs), in the body. Further, due to this defective ARG1 enzyme, ornithine levels are generally low in patients with ARG1-D, and the urea cycle is impaired.

Persistently elevated levels of arginine and its toxic metabolites called the Guanidino compounds (figure 1), are associated with neurotoxic and hepatotoxic disease manifestations and progressive motor function decline in animal models and patients with ARG1-D. Moreover, ARG1-D leads to an excess of toxic NO (nitrous oxide), which also contributes to demyelination as shown *in-vitro,* by mitochondrial dysfunction of the oligodendrocytes.

Through gene expression profiling of the motor cortex of an ARG1-D mouse knockout model, pre-clinical models have shown that arginase-1 deficiency causes demyelination of the corticospinal tract during postnatal central nervous system development, with altered synapse density in the motor cortex.

According to some case reports, reductions in plasma arginine and its metabolites via a protein-restricted dietary therapy led to lowering of CSF arginine concentrations and a reduction of symptoms. There are several case reports where liver transplantation completely ameliorated hyperargininaemia (and hyperammonaemia) and disease progression in ARG1-D patients.

2.1.4. Clinical presentation and diagnosis

Signs and symptoms

Neuromotor complications are a hallmark feature of ARG1-D, and the lower-limb spasticity typically seen in early childhood impairs mobility and balance, leading to difficulties in walking and climbing stairs. Patients typically present with some form of lower-limb spasticity, with approximately 60% to 75% having spasticity at initial presentation, which increases with extended follow-up. Progressive spastic diplegia results in significant effects on mobility and morbidity. These neuromotor manifestations of the disease (e.g., spastic diplegia) are distinct from the known neurotoxic effects of hyperammonaemia (e.g., psychosis, altered mental status, cognitive and learning deficits, and seizures), and unique relative to other UCDs, supporting a distinct pathophysiology from other UCDs. Other common symptoms are nausea and vomiting, failure to thrive and growth retardation, and hepatic impairment with liver function test increments.

Hepatomegaly and hepatic impairment commonly occur. About 50-60% of the patients have concurrent episodes of hyperammonaemia, although these occur with later onset and are less severe than in other UCD. Epilepsy is common (about 30-50%) but often manageable.

Disease course

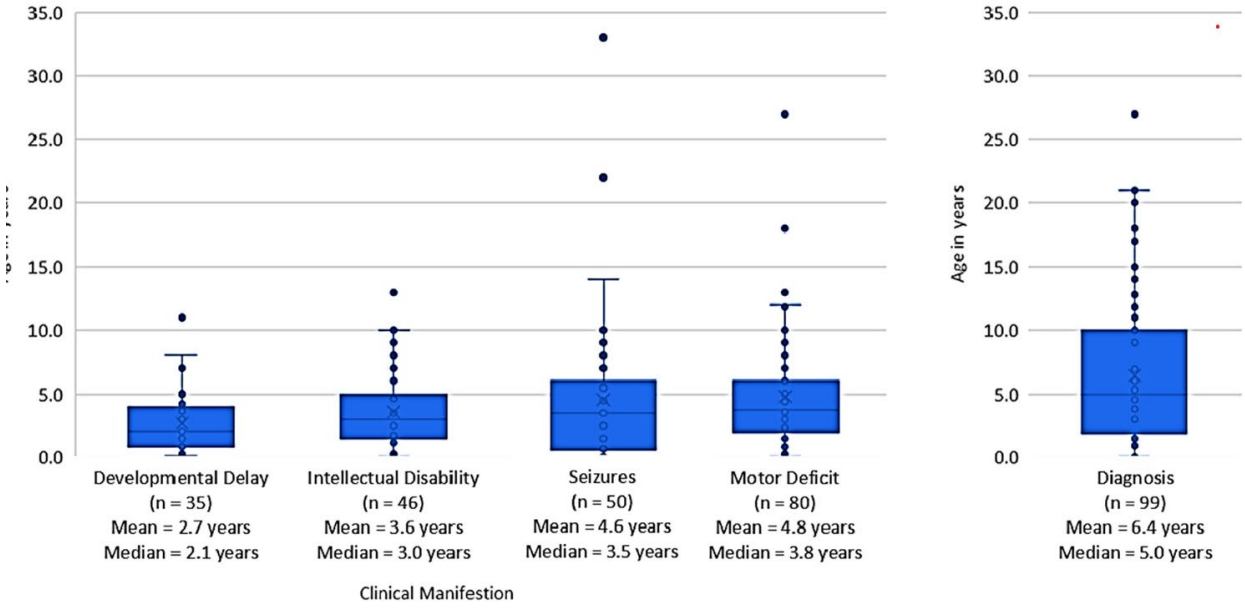
Unlike other UCDs where many patients present in the first days of life with severe hyperammonaemia, most ARG1-D patients are asymptomatic at birth through early infancy. Typically, patients develop initial symptoms at 2 to 3 years of age. A systemic review of the natural history of 157 cases described in the literature is of interest. According to this review, the diagnosis was often delayed (mean 6.4 y), while developmental and cognitive delay and motor deficits occurred earlier (Figure 2).

Variation in timeframe and progression of symptoms has been observed between patients.

There were too limited data to establish median survival, which is shortened by weakened state, liver function impairment or seizures. Some individual cases are described of patients surviving to in their forties, suffering from painful contractures and mental deficits.

No data on mortality were provided.

Figure 2 Age of onset of clinical manifestations and diagnosis (source Bin Sawad, 2022)



Diagnosis

Diagnosis can be readily made with routinely available assessment of red blood cell arginase levels, plasma arginine assessment, or genetic counselling. The diagnosis of arginase deficiency is either confirmed by the identification of biallelic pathogenic variants in ARG1 genome, or by low arginase enzyme activity (usually <1% of normal) in red blood cell extracts.

Delay in diagnosis may occur due to the overlap in symptomatology with other developmental diseases, such as cerebral palsy or hereditary spastic paraplegia, and lack of disease awareness.

ARG-1 deficiency is not part of routine genetic screening in neonates in Europe.

2.1.5. Management

Current management approaches for ARG1-D mainly consist of dietary protein restriction to reduce arginine, together with essential amino acid (EAA) supplementation. International guidelines for ARG1-D focus on the reduction of plasma arginine to levels of <200 µM and ideally within the normal range (defined as 40 to 115 µM) as the primary treatment goal.

However, dietary modification can produce modest reductions in plasma arginine levels. Reducing plasma arginine to the guideline-recommended level of <200 µM is difficult to achieve via dietary restriction alone as arginine flux is largely dependent on whole-body protein turnover and is minimally affected by dietary intake. It has been estimated that about 20-25% of the natural human arginine is derived from the diet. In addition, the diet is unpalatable and difficult to maintain and manage, especially in growing children, resulting in poor compliance.

Other treatments, such as ammonia scavengers, help to control excessive ammonia levels. In addition, spasmolytic agents and anti-epileptics are applied as symptomatic treatments. Still, ammonia scavengers only target a part of the symptoms, i.e. those related to (acute) hyperammonaemia. However, they do not reduce the formation of the toxic metabolite of arginine, which causes the neurotoxicity that is the hallmark of the disease (spasm and motor disorders).

Liver transplantation has been reported to normalise arginine and ammonia levels and ameliorate neurological symptoms in individual cases, and significantly improve neurodevelopmental and growth delay. At the same time, liver transplantation is available to only a small fraction of patients and carries a significant risk of morbidity and mortality, also related to life-long required use of immunosuppressant agents.

2.2. About the product

Mode of action

Pegzilarginase is a modified, cobalt-substituted, PEGylated recombinant human ARG1 enzyme with enhanced stability, more potent catalytic activity, and an extended half-life compared to the native enzyme. In concept, the product is enzyme therapy for patients with ARG1-D by substituting for the deficient human ARG1 enzyme activity in these patients.

Approved indication

The therapeutic indication is: *Loargys is indicated for the treatment of arginase 1 deficiency (ARG1-D), also known as hyperargininemia, in adults, adolescents and children aged 2 years and older*.

Approved posology

The recommended initial dose of Loargys is 0.1 mg/kg per week, either administered intravenously or sub-cutaneous. The dose may be increased or decreased in 0.05 mg/kg increments to achieve therapeutic goals. Doses above 0.2 mg/kg per week have not been studied in clinical trials in ARG1-D.

Prior to initiating treatment, a baseline plasma arginine concentration should be obtained. After initiating treatment, the weekly dose should be adjusted based on pre-dose plasma arginine concentrations to maintain plasma arginine within the normal range. To maximise the time within the normal range, dose adjustments should be aimed at achieving a pre-dose level of plasma arginine near the upper limit of normal (ULN). The dose adjustment should typically be based on two consecutive measurements, and first such assessment performed after 4 weeks of administration. Monitoring plasma arginine levels weekly for 2 weeks after any dose adjustment is recommended to assess impact of the dose change.

Once the individualised dose level has been established, monitoring of plasma arginine concentration is recommended to be performed in accordance with standard clinical monitoring visits. Validated methods to monitor arginine levels are to be used in patients treated with Loargys, as standard methods are not adequate to control residual enzyme activity of pegzilarginase after sampling, and may lead to artificially low arginine levels, and incorrect dose adjustments.

2.3. Type of application and aspects on development

The applicant requested consideration of its application for a Marketing Authorisation under exceptional circumstances in accordance with Article 14(8) of the above-mentioned Regulation based on the inability to provide comprehensive clinical data on the efficacy and safety under normal conditions of use, due to the rarity of the ARG1-D condition.

Moreover, due to the slowly progressing nature of the disease it is reasonable to expect that in order to demonstrate statistically compelling effects, clinical trials of longer duration would be required. Such placebo-controlled trials are not considered feasible due to ethical considerations.

2.4. Quality aspects

2.4.1. Introduction

The finished product is presented as solution for injection/infusion containing 5 mg/mL of pegzilarginase as active substance.

Other ingredients are: sodium chloride, potassium dihydrogen phosphate, dipotassium phosphate, glycerol, hydrochloric acid (for pH adjustment), sodium hydroxide (for pH adjustment) and water for injections.

The product is available in type 1 glass vial with a stopper (coated chlorobutyl elastomeric rubber) and a flip off seal (aluminium) in carton box. Each carton contains a 3 mL vial (fill volume of 0.4 mL and a blue flip-off cap) or a 5 mL vial (fill volume of 1.0 mL and a white flip-off cap).

2.4.2. Active Substance

2.4.2.1. General information

Pegzilarginase (INN) is a covalently PEGylated recombinant human arginase 1 (rhARG1) with cobalt replacing the naturally present manganese metal cofactor. The rhArg1 is a homotrimer produced in *E. Coli*. The monomer of rhArg 1 is missing the N-terminal methionine found in the native human arginase 1 (hArg1) monomer. The substitution of the native manganese (Mn^{2+}) with cobalt (Co^{2+}) in the active site of arginase 1 is intended to enhance the *in vivo* half-life and catalytic activity at physiological pH. The structure of rhArg 1 before PEGylation and selected properties of the PEGylated protein are presented in the dossier. The total molecular weight of pegzilarginase active substance ranges between 224-344 kDa.

2.4.2.2. Manufacture, process controls and characterisation

The active substance is manufactured, tested and released at the following facilities, in accordance with Good Manufacturing Practice (GMP). The commercial active substance manufacturing site is Fujifilm Diosynth Biotechnologies U.S.A. Inc., 6051 George Watts Hill Drive, 27709 North Carolina, United States.

Description of the manufacturing process and process controls

The pegzilarginase active substance manufacturing process has been adequately described. The primary container is stated to comply with the USP <88> Class VI requirements and with Commission Regulation EU 10/2011 (and amendments) on plastic materials and articles intended to come into contact with food. The provided information is considered acceptable. The applicant committed to revise the specification for the active substance container by including additional tests (Recommendation).

The upstream process consists of inoculum expansion and fermentation. The resulting cell slurry can be stored frozen at ≤-60°C until further processing. The downstream process consists of several steps that are described in the dossier, consisting of different chromatography techniques, cobalt substitution, ultrafiltration/diafiltration, PEGylation and bulk fill. The pegzilarginase manufacturing process does not include any reprocessing steps. Overviews of the upstream and downstream manufacturing processes are provided in the dossier.

A flow diagram, narrative descriptions and tabular overviews of process parameters, operational ranges, and in-process controls (IPCs) are provided for each process step. Critical parameters and controls are not included in these overviews, but listed separately in section 3.2.S.2.4. As appropriate references are included in section 3.2.S.2.2, this can be accepted.

The selection of process parameters included in 3.2.S.2.2 and their operational ranges are, in general, supported by process development and validation studies. However, a Major Objection was raised during the assessment, concerning the insufficient proof for ensuring a consistent performance of the PEGylation step. In response, the applicant has provided further detail on the process development data that had been submitted and additional data to support the upper and lower limits for reaction time. Overall, the control strategy for the PEGylation step is now considered sufficiently justified and therefore this issue is solved.

The manufacture of the methoxy PEG succimidyl carboxymethyl ester (mPEG intermediate) from the starting material has also been described and is considered adequate.

Control of materials

The applicant has provided an overview of the raw materials, resins, filters, membranes, culture medium and buffers used in the manufacture of pegzilarginase active substance. Compendial raw materials are tested in accordance with the corresponding monograph, while specifications (including test methods) for non-compendial raw materials are presented. For the chromatography columns, regulatory product information files are provided. The composition of the culture media, feed solution, induction solution, buffer composition and ready-to-use solutions of kanamycin, glucose and potassium phosphate are also provided.

No materials of animal origin are used in the generation of the master cell bank (MCB), WCB or the expansion and production media, except L-cysteine hydrochloride monohydrate used in fermentation and shake flask media. The L-cysteine certificate of analysis is provided for reference. The use of L-cysteine is evaluated to be a negligible contribution to the risk of transmissible spongiform encephalopathies (TSE). Glycerol is used as an excipient and may be derived from beef tallow. A statement of compliance with EMA/410/01 rev 3 provided by the active substance manufacturer is included in the dossier.

Overall, sufficient information on raw materials used in the active substance manufacturing process has been submitted.

Starting material

The methoxy PEG succimidyl carboxymethyl ester was initially designated as critical raw material. As this material already contains the functional group to link the PEG to the enzyme, this designation was not agreed and a Major Objection was raised during the procedure to reclassify it as critical intermediate. In response, the starting material has been re-defined and is regarded as an acceptable starting material in view of ICH Q11 (considering the number of steps from API and the fact that it is a well-characterised material that is available as a commodity in the non-pharmaceutical market). The route of synthesis of the starting material is depicted in the dossier. The specification for the starting material has been provided and it is considered acceptable.

Cell banks

The applicant has provided sufficient information on the expression construct, including the expression plasmid map and DNA sequence.

The plasmid was used to transform commercially available *E. coli* cells. Sufficient information is provided on the characterisation of the MCB and WCB, in line with the relevant ICH guidelines. Stability

of the MCB and WCB was adequately evaluated. The stability test plan for MCB and WCB and the approach to qualifying future WCBs are acceptable.

Testing of End of Production Cells (EoPC) included plasmid DNA sequence, plasmid retention in the cell line, gene copy number analysis, restriction enzyme analysis and arginase expression. The applicant committed to perform EoPC testing for the presence of bacteriophage as part of characterisation of the current WCB (Recommendation).

In conclusion, sufficient information is provided regarding testing of MCB and WCB and release of future WCBs. Genetic stability has been demonstrated for cells at and beyond the limit of cell age.

Control of critical steps and intermediates

The applicant has provided information on critical process parameters and their operational ranges, IPCs and acceptance criteria, IPCs methods, in-process intermediates and hold times. Actions taken if limits are exceeded are specified. The acceptance criteria for the IPCs are acceptable and the provided method validations give no reason for concern.

The applicant lists several intermediates that may be stored prior to further manufacturing.

Tests and acceptance criteria for the mPEG intermediate are laid down in the dossier. The mPEG intermediate specification includes tests for critical aspects and are considered acceptable. The characterisation and elucidation of the structure of the mPEG intermediate, including impurity risk assessment and control strategy of the mPEG related impurities are also described in the dossier and found acceptable. The claimed absence of possibly carcinogenic side products as residual impurity has been sufficiently substantiated. The applicant committed to complete the method validation for mPEG intermediate (Recommendation).

In conclusion, acceptable information has been provided on the control system in place to monitor and control the pegzilarginase active substance manufacturing process.

Process validation

The applicant performed a Process Performance Qualification (PPQ) study with three consecutive batches. The majority of PPQ acceptance criteria for process parameters and in-process controls were met. Deviations were sufficiently discussed and do not give rise to any concern. The PPQ results demonstrate that the process can consistently operate within the predefined process parameters ranges. Batch analysis results of the PPQ batches showed no major differences and all active substance release criteria were met. No specific studies have been done to demonstrate the removal of impurities, however PPQ results confirm consistent control of impurities. Reprocessing has not been validated as there are no reprocessing steps claimed by the applicant.

Column resin lifetime studies were performed to support the repeated use of the chromatography process pre-packed GE Column based on protein carryover. Effective sanitisation was demonstrated. A clear definition of the resin lifetime and the approach used for confirming column lifetime during commercial manufacturing has been included in the dossier and is considered acceptable.

The extractables/leachables evaluation was based on a risk assessment. Based on this assessment, studies were limited to an extractable study for the active substance container. No extractables were identified after 7 days of gentle agitation at room temperature. This approach is considered sufficiently justified.

Hold time validation studies were performed. The results support the proposed in-process hold durations. The drug (active) substance intermediate (DSI) and the active substance generated from the cumulative hold study met all specifications.

A shipping validation study has also been performed, indicating that no product impact is expected after shipping.

In conclusion, the pegzilarginase active substance manufacturing process is considered adequately validated.

Manufacturing process development

Comparability

During development, the active substance has been manufactured at four different manufacturing sites, including the commercial manufacturing site Fujifilm Diosynth (FDBU). A comparability assessment has been provided for the active substance manufactured at the latter three sites. This is acceptable, as the batches produced at the first site were not included in clinical studies.

The comparability assessment consists of an overview and risk assessment of the process changes, a retrospective comparison of batch analysis data, a comparison of impurity data, stability data and characterisation data. No major changes have been introduced to the manufacturing process.

Comparison of batch analysis data includes data from the three different sites. Some differences between batches manufactured at the different sites are noted. Partly, these could be regarded as an improvement (i.e. lower levels of impurities) and can be accepted. The observed variability in enzyme activity appears to be unrelated to site-to-site differences and, following a Major Objection raised during the assessment, the applicant has sufficiently justified that the observed variability can be attributed to assay variability. This conclusion is endorsed, and the Major Objection considered solved. A higher PEG:protein ratio for the active substance batches manufactured at the first site compared to the batches manufactured at the second and third (commercial drug substance site) was also observed. As a similar difference is observed in the batch analysis data of the clinical finished product batches manufactured from these active substance batches, a separate Major Objection was raised and the applicant was asked to further justify that this difference has no impact on the kinetics, safety and/or efficacy of the product, as well as to demonstrate that consistent control of the active substance/finished product can be ensured. The applicant justified that the observed differences are also attributable to method variability. In addition, a new method for determination of the PEG:protein ratio was introduced, ensuring a more reliable control of this parameter. The Major Objection that was raised on this issue is, therefore, considered solved.

Elements of physicochemical properties and primary, secondary and higher-order structure have been characterised and are presented in a separate comparability report. Substantial batch-to-batch variation in PEGylation sites was observed when comparing the batches from the different sites. Therefore, this issue was raised as part of the Major Objection concerning the control of the PEGylation step. In addition, data suggest that peptide mapping results of some of the batches do not comply with the active substance specification. Considering also the observed variability in enzyme activity, PEGylation profile and the divergent imaged capillary isoelectric focusing (icIEF) pattern observed in the corresponding two finished product batches, a separate Major Objection was raised, requesting the applicant to justify the observed variability. The applicant indicated in its response that the differences observed in PEGylation sites/occupancy and in peptide mapping results can be attributed to differences in the methods used. This conclusion is endorsed, and both these issues are therefore considered solved. The comparability of the two early batches used in clinical studies remained, however, questionable. Based on the provided data, it is nevertheless considered unlikely that this has impacted the outcome of the clinical studies and this issue was therefore not further pursued.

Stability data are provided for two batches from each site used for clinical batches and seven batches from FDBU for up to 24 or 12 months at long-term storage conditions (<-60°C) and 3 months at accelerated conditions (5°C). These data do not give rise to concerns.

Process development

The information to support the process design and control strategy includes summaries of process optimisation studies, results of technology transfers from the different sites (including scale-up), and process characterisation studies performed at FDBU that included a Failure Modes and Effects Analysis (FMEA) to identify risk, one factor a time (OFAT) and Design of Experiments (DOE) studies. In addition, an overview of all Quality Attributes identified during development, their criticality assignment and control strategy is provided. The general approach for process evaluation/characterisation can be accepted.

The selection of critical quality attributes (CQAs) is considered appropriate.

Characterisation

The applicant has characterised the structure and other characteristics of pegzilarginase active substance with a broad range of analytical techniques. Results are in general consistent with the proposed structure.

Furthermore, heterogeneity of the active substance was adequately characterised. The studies performed to evaluate (forced) degradation are overall sufficient. In addition, the applicant has provided a clear overview of the potential process-related impurities, for which active substance release acceptance criteria are in place.

In summary, the characterisation is considered appropriate for this type of molecule.

2.4.2.3. Specification

The quality control specification for pegzilarginase active substance is provided. The proposed active substance release test panel includes tests for general parameters, identity, quantity and biological activity, tests for purity/product-related impurities, tests for process-related impurities, microbial controls, residual manganese, PEG:protein ratio. During the assessment, a test for free sulfhydryl, with acceptable criteria, has also been introduced upon request. Part of the tests is performed on the DSI prior to PEGylation. This can be accepted.

The specification was established based on regulatory guidelines, analytical capability, process capability and experience. The applicant initially set the quantitative active substance acceptance criteria based on the tolerance interval of the combined release data from eleven GMP active substance batches and fourteen GMP finished product batches. As the formulation of the active substance and finished product is essentially the same, it is agreed that both active substance and finished product data can be taken into account. Considering the observed differences between batches manufactured at one of the manufacturing sites used for clinical batches and at the commercial active substance manufacturing site, it was however not agreed to use data from batches produced at this site for calculating the tolerance intervals to support the commercial active substance and finished product acceptance criteria for several quality attributes. Upon request, these acceptance criteria have been revised/tighten by the applicant. In response to the Major Objection on control of PEG:protein ratio, the applicant has introduced a new method to control this parameter and the applicant was asked to further tighten the specification for PEG:protein ratio, in line with the tolerance interval calculated from the batch analysis data. It is noted that this range is wider than the PEG:protein ratio observed in clinical batches. However, as the clinical data show no correlation between the degree of PEGylation and plasma-arginase levels, this is considered acceptable.

1*Analytical methods*

The applicant has provided descriptions of all methods, including, if relevant, information on the method principle, critical reagents and equipment, sample preparation and dilutions, reference standards and controls, procedural details, system suitability and sample acceptance criteria, and data analysis and reportable results. Potency is controlled by a specific activity assay.

The provided information on the analytical methods is in general sufficient, including for the newly developed method for determination of PEG:protein ratio. Non-compendial methods have been validated in line with ICH Q2(R2). Validation was performed at the commercial testing site. Most methods were validated using DSI and/or active substance. It is agreed that the validation results for the active substance are considered representative also for the finished product.

Batch analysis

Batch analysis data cover all development, clinical, toxicity, stability and three validation (PPQ) batches. In total, data are provided for fifteen active substance batches manufactured at the different active substance manufacturing sites used. Batch results show, in general, sufficient consistency, but substantial variation is observed in enzyme activity and some differences are noted between batches manufactured at the different active substance manufacturing sites. This is further discussed in the active substance section on comparability.

Reference materials

The applicant has provided an overview of the primary reference standards (PRS) used to date, including information on manufacturing, characterisation and stability. The reference standards are derived from active substance batches or DSI batches. Characterisation and qualification included all necessary tests, including mapping of peptides. Each next PRS is going to be qualified against the current one. Appropriate criteria for expiration and possible re-testing and re-qualification of reference standards are defined. Overall, the information is considered acceptable.

2.4.2.4. Stability

The proposed shelf-life for pegzilarginase active substance is 36 months at ≤-60°C. Stability studies to support the proposed shelf-life were performed in line with ICH Guidelines.

The stability test panel is presented and is considered appropriate and includes methods that were demonstrated to be stability-indicating. The containers used in the primary stability studies are a scale-down model of the 5 L bottles used in routine storage and are considered representative for the commercial container closure system (CCS).

Seven batches from the commercial site FDBU were included in the stability studies at ≤-80°C (48 months), 5±3°C (1-3 months), 25°C/60% relative humidity (1 month) and 40°C/75% relative humidity (2 weeks, 1 batch). Long-term stability data are available for storage up to 36 (n=4) or 24 (n=3) months. Stability studies with two batches from one of the manufacturing sites for clinical batches (24 and 48 months long-term data) and two batches from one of the other manufacturing sites for clinical batches (36 months long-term data) are provided as supportive studies. No trends were observed in the parameters studied at the proposed long-term storage temperature.

In summary, the provided stability data are considered supportive and the proposed shelf-life of 36 months at ≤-60°C when stored in the declared CCS is endorsed.

2.4.3. Finished Medicinal Product

2.4.3.1. Description of the product and pharmaceutical development

The finished product is presented as a sterile solution containing 5 mg/mL of pegzilarginase as active substance formulated with sodium chloride, monobasic potassium phosphate, dibasic potassium phosphate and glycerol with a target pH of 7.4. The finished product qualitative and quantitative compositions are presented in the dossier.

The product is available in a 5 mL Type 1 glass vial with 1 mL fill and in a 3 mL Type 1 glass vial with 0.4 mL fill. Chlorobutyl elastomeric stoppers coated with Teflon® (for the 3 mL vial) or Flurotec® (for the 5 mL vials) are used. The material complies with Ph. Eur. and EC requirements. The choice of the container closure system has been validated by stability data and is adequate for the intended use of the product. The choice of container closure system has been sufficiently described. Extractables and leachables studies were performed. In general, the assessment is considered comprehensive and do not give raise to any concerns. Additional extractables and leachables stability studies will be included with the planned stability studies for one of the process performance qualification batches of the 1 mL fill and one batch of the 0.4mL fill and will include non-volatile, semi-volatile and volatile leachable testing. This approach is endorsed.

There are no overages utilised in the finished product manufacturing process. The commercial finished product has an excess volume that allows for the withdrawal of the labelled volume. The target excess volume for both the 0.4 mL and 1 mL presentations is 0.1 mL. Labelling of the pegzilarginase content is based upon the un-PEGylated enzyme, which is in line with the applicable guidelines.

The pharmaceutical development of pegzilarginase injection focused on the selection of a formulation capable to ensure suitable physicochemical and biological properties, targeted to achieve the desirable safety and efficacy, considering the routes of administration, as well as product stability. Sufficient information is provided on the active substance, which is formulated with the same excipient concentration as the finished product. Selected excipients are fit for purpose and compatibility between the excipients and the active substance is considered demonstrated by the long-term stability data. All excipients comply with Ph. Eur. No novel excipients or excipients of human origin are used. As discussed in the active substance section, as glycerol may be derived from beef tallow, a statement of compliance with EMA/410/01 rev 3 is provided in the dossier and is considered acceptable.

The finished product is intended for administration by intravenous infusion (following dilution with sterile 0.9% sodium chloride solution) or by subcutaneous injection (used undiluted). The product is supplied without diluent. The applicant performed compatibility studies using commonly used syringes and needle (cannula) combinations for both the undiluted and the diluted with normal saline finished product, at 2°C to 8°C and room temperature. The results of these studies support the claimed chemical and physical in-use stability for the finished product after preparation for up to 4 hours at 2°C to 8°C or up to 2 hours at room temperature prior to administration.

The applicant has provided an overview of the changes made to the finished product manufacturing process during development. The main changes include the protein concentration, active substance source (manufacturing sites), vial size, stopper size, stopper coating and fill volume. No changes were made to the buffer composition during the clinical development and for the commercial product. In clinical trials, a 1 mg/mL presentation and a 5 mg/mL presentation have been used, however the 1 mg/mL presentation will not be commerciald.

An overview of finished product batches is provided, which includes in total sixteen batches. Six PPQ batches have been manufactured according to the proposed commercial process with a protein

concentration of 5 mg/mL and a fill volume of 0.4 mL or 1 mL. The date of manufacture, batch size, active substance manufacture and use of each batch is specified.

The applicant evaluated the comparability of the finished product batches during development by a retrospective analysis of analytical results. In addition, stability data are provided to demonstrate that the difference in headspace and stoppers does not impact the quality of the finished product. The analytical results show some differences between finished product batches manufactured using active substance batches from the commercial manufacturing site FDBU and finished product batches manufactured using active substance from the manufacturing site used for earlier clinical batches. This point was raised as Major Objection during the assessment and considered solved following additional justification provided by the applicant (reference is made to the active substance section on comparability).

In conclusion, adequate justification is provided for the finished product formulation and manufacturing process development.

2.4.3.2. Manufacture of the product and process controls

The pegzilarginase finished product is manufactured, tested and packaged in accordance with GMP. Batch release of the finished product is performed by Unimedic AB, Storjordenvägen 2, 864 31 Matfors, Sweden and by Immedica Pharma AB, Solnavägen 3H, 113 63 Stockholm, Sweden.

A flowchart and brief narratives of each process step are provided. The manufacturing process of the finished product is considered standard and has, in general, been sufficiently described. Briefly, it consists of active substance thaw, concentration adjustment, filtration through two redundant sterilizing grade filters, aseptic filling, stoppering and capping. There are no reprocessing steps in the finished product manufacturing process. The proposed manufacturing scale is supported by the PPQ study.

Control of critical steps and intermediates

The applicant provided information on process parameters, IPCs and in-process hold times. No isolated intermediates are defined in the finished product manufacturing process. The process parameters are laid down in the dossier. The selection and acceptance criteria of IPCs are appropriate and sufficient information is provided on the control methods and their validation. The applicant committed to implement a pre-filtration filter integrity test (PUPSIT) prior to production of the next finished product lot (Recommendation).

The proposed in-process hold times and total processing time from the start of formulation to storage of the finished product are also acceptable.

Process validation and/or evaluation

The applicant has provided summarized information on the PPQ, media fill studies, sterilizing filter validation, product-contact equipment sterilization, hold time studies, container closure integrity qualification and shipping validation.

A FMEA was performed to evaluate the impact of process parameters on process performance and CQAs (reference is made to the active substance section on process development). The selection of process parameters included in the manufacturing process description and their operational ranges are sufficiently justified.

Three PPQ batches have been manufactured for the 0.4 mL finished product presentation and four for the 1 mL finished product presentation. However, one of the latter batches did not meet the criterion

for container content of not less than 1.0 mL and, therefore, it was considered not acceptable as a PPQ batch and will not be released for commercial distribution. However, the batch is still considered demonstrative of the aseptic process and was also included in the stability program. This approach is endorsed.

The PPQ batches were manufactured at the proposed commercial scale following the proposed commercial manufacturing process. The performed in-process tests and acceptance criteria are in line with the IPCs listed in the dossier and all PPQ batches complied with the results of in-process testing. The finished products of the PPQ batches were sampled at the beginning, middle and end of filling and tested in accordance with the proposed finished product release test panel. All three of 0.4 mL fill and three of 1 mL fill of 5 mg/mL PPQ batches met the acceptance criteria and the results are consistent between the batches and the different stages of filling.

The results of the filter validation support the conclusion that the membrane filters and operational limits are suitable for sterilizing the finished product. Depyrogenation and sterilization of the vials is sufficiently validated.

The proposed in-process hold times and total processing time from the start of formulation to storage of the finished product are supported by media fill studies and data from a hold time study performed with dilution buffer and a pooled active substance (n=1). Limited information on the hold time study is provided, but considering the short processing time, no further questions were raised.

In conclusion, the finished product manufacturing process can be considered validated. It has been demonstrated that the manufacturing process is capable of producing the finished product of intended quality in a reproducible manner.

2.4.3.3. Product specification

The quality control specification for pegzilarginase finished product is provided and is identical for the 5 mg/mL 1 mL fill and the 5 mg/mL 0.4 mL fill, except for the difference in the container content. The proposed finished product release test panel is acceptable, and it is set in accordance with the principles defined in ICH Q6B. It includes tests for general parameters, identity, quantity, biological activity, purity/product-related impurities, microbial controls, as well as product specific parameters as detailed in the dossier.

Because the active substance and finished product are both formulated at 5 mg/mL in the same buffer, a common set of acceptance criteria are proposed for those tests performed on both active substance and finished product. Test for process-related impurities are also performed at the active substance stage. Questions raised with regard to the active substance acceptance criteria for several quality parameters were also applicable to the finished product acceptance criteria (reference is made to the active substance section on specification). Tests that are specific for the finished product include sterility, subvisible particulates, container closure integrity, container content and osmolality. In conclusion, the acceptance criteria are endorsed and ensure an adequate control of the quality of the finished product. **2**

No additional process or product-related impurities are introduced or expected to form as a result of the finished product manufacturing. Therefore, finished product impurities are expected to be the same as those described in the active substance section.

A risk evaluation concerning the presence of nitrosamine impurities in the finished product has been performed considering all suspected and actual root causes in line with the "Questions and answers for marketing authorization holders/applicants on the CHMP Opinion for the Article 5(3) of Regulation (EC) No 726/2004 referral on nitrosamine impurities in human medicinal products" (EMA/409815/2020) and

the "Assessment report - Procedure under Article 5(3) of Regulation EC (No) 726/2004 - Nitrosamine impurities in human medicinal products" (EMA/369136/2020). Based on the information provided it is accepted that no risk was identified on the possible presence of nitrosamine impurities in the active substance or the related finished product. Therefore, no additional control measures are deemed necessary.

An elemental impurities risk assessment has also been presented. All elemental impurities were below the ICH Q3D permitted daily exposure (PDE) and control threshold levels, and no further testing is required. In addition, the risk of extractable and leachables is found sufficiently addressed and did not give rise to any questions.

Analytical methods

Most analytical procedures are either identical to those described for the active substance or compendial. Brief method descriptions are provided for those methods that have not yet been described in the active substance section and the provided information is sufficient. The only non-compendial method which is unique to finished product is the container closure integrity testing (CCIT), for which appropriate validation data in accordance with ICH guidelines have been provided.

Batch analysis

Batch analysis data are provided for ten finished product batches of 5 mg/mL at the commercial scale and six finished product batches of 1 mg/mL, manufactured using active substance batches supplied by two different active substance manufacturing sites used during development. In general, batch results show sufficient consistency, however some differences are noted depending on the origin of the active substance. This is further discussed in the section on manufacturing process development.

Reference materials

Reference is made to the corresponding active substance section.

2.4.3.4. Stability of the product

The proposed finished product shelf-life is 24 months at 2°C to 8°C, protected from light. Stability studies to support the proposed shelf-life were performed in line with ICH Guidelines. The proposed stability test panel is considered appropriate and includes stability-indicating methods. Stability studies are performed at -80±10°C (48 months), 5±3°C (36 months), 25°C/60% relative humidity (6 months) and 40°C/75% relative humidity (3 months). The containers used in the primary stability studies are stated to be identical to the indicated finished product container closure system.

All manufactured finished product batches have been included in stability studies and all batches have been manufactured at the intended commercial manufacturing site. For the supportive batches (n=12), up to 36 months long-term stability data are available. For the primary PPQ stability batches (n=3 for the 0.4 mL fill and n=3 for the 1 mL fill), between 3 to 12 months of long-term stability data are available. Overall, no trends were observed in the parameters studied at the proposed long-term storage temperature.

Additionally, an ICH photostability study using finished product filled in the commercial primary and secondary packaging have been provided. It has been concluded that the product is photostable when stored in the proposed commercial packaging.

Overall, based on the currently available long-term stability data, the proposed shelf-life of 24 months for the finished product stored at 2-8°C in the commercial carton packaging can be endorsed. In addition, the in-use storage conditions as proposed in the SmPC for the unopened vial (*Once removed*

from the refrigerator, Loargys can be stored for 2 hours at room temperature up to 25°C) and for the finished product after preparation (*Chemical and physical stability has been demonstrated for 2 hours when stored at room temperature up to 25 °C or up to 4 hours if stored refrigerated at 2 °C to 8 °C. If the product is not used within these time frames, it must be discarded. From a microbiological point of view, the product should be used immediately after preparation*) are considered justified by the in-use compatibility studies.

2.4.3.5. Post approval change management protocol(s)

The applicant initially submitted a post-approval change management protocol (PACMP) for the removal of kanamycin used in the fermentation step. However, following questions raised during the assessment, the PACMP was withdrawn by the applicant.

2.4.3.6. Adventitious agents

Microbial contamination
Bioburden and endotoxin are monitored at several steps during the manufacturing process. The finished product is controlled tested for sterility and endotoxin at release and during shelf-life.

TSE
Glycerol may be derived from beef tallow. A statement of compliance with EMA/410/01 rev 3 is provided and it is deemed acceptable. No other materials of animal or human origin are used in the manufacturing process.

Adventitious viruses
As the product is produced in *E. coli*, there is no need for testing for endogenous or adventitious viruses or for the introduction and validation of virus clearance steps.

2.4.3.7. GMO

Not applicable.

2.4.4. Discussion and conclusions 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 Quality by Design (QbD) principles in the development of the active substance and/or finished product and their manufacturing process. However, no design spaces were claimed for the manufacturing process of the active substance, nor for the finished product.

Several Major Objections were raising during the assessment concerning: 1) insufficient proof for demonstrating consistent performance of the PEGylation step; 2) insufficient proof that the proposed release method and acceptance criterion for determination of the PEG:protein ratio can ensure consistent control of the active substance/finished product; 3) redefinition of the methoxy PEG succimidyl carboxymethyl ester as an intermediate instead of starting material; 4) insufficient proof for demonstrating consistent process performance and control as highlighted by the observed batch-to-

batch variability for several CQAs. All of these mentioned concerns have been adequately addressed by the end of the procedure.

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, which pertain to: further validation of the method for mPEG intermediate, implementation of a pre-filtration filter integrity test prior to next production run of the finished product, EoPC analysis for the presence of bacteriophage as part of characterization of the current WCB and revision of the specification for the active substance container by including additional tests. These points are put forward and agreed as recommendations for future quality development.

2.4.5. Conclusions on the chemical, pharmaceutical and biological aspects

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

2.4.6. Recommendations for future quality development

In the context of the obligation of the MAHs to take due account of technical and scientific progress, the CHMP recommends the following points for investigation:

1. The applicant commits to complete the method validation for mPEG intermediate.
2. The applicant commits to implement a pre-filtration filter integrity test (PUPSIT) prior to production of the next pegzilarginase finished product lot.
3. The applicant commits to analyze EoPC for the presence of bacteriophage as part of characterization of the current WCB.
4. The applicant commits to revise the specification for the active substance container by including additional tests.

2.5. Non-clinical aspects

2.5.1. Introduction

The non-clinical program was designed to characterize the pharmacology, pharmacokinetics (PK), and toxicology of pegzilarginase in support of intravenous and subcutaneous administration in patients with ARG1-D. All pivotal non-clinical studies conducted under the program to investigate the safety of pegzilarginase were performed in accordance with applicable International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH), Committee for Medicinal Products for Human Use (CHMP), and FDA guidance documents (e.g., ICH M3 (R2), S6, S7A, S7B, and S9).

Initial *in vivo* efficacy studies in mouse models mimicking the ARG1-D disease state were used in early studies. The intraperitoneal (IP) route of administration was initially utilized to assess the pharmacologic effects of pegzilarginase on arginine levels. These models, with significant excesses of circulating arginine and catabolites of arginine, differ from human ARG1-D in that mice exhibit severe and generally lethal hyperammonaemia (Iyer et al., 2002; Kasten et al., 2013; Burrage et al., 2015), a complication likely complicating survival evaluation. Rats were also studied following intravenous (IV) administration of pegzilarginase to assess the pharmacologic effects in an arginine-induced model of

hyperargininaemia (Adriaenssens at al., 1984). *In vivo* pharmacology studies in neonatal transgenic mouse and adult tamoxifen-induced mouse models of hyperargininaemia utilized the IP route of administration to assess the pharmacological effects of pegzilarginase on arginine levels.

The analysis of the PK profile was evaluated following the subcutaneous (SC) or IV route of administration. Because of the direct connection between the PD effect on arginine and the pharmacological findings, a refined PD collection method using the known arginase inhibitor Nω-hydroxy-nor-Arginine (nor-NOHA) in the blood collection tubes was incorporated in later non-clinical studies to prevent pegzilarginase degradation of arginine and to provide an accurate assessment of arginine modulation. The PK properties were comparable across all studies regardless of the presence of nor-NOHA. Immunogenicity was assessed to determine the incidence and extent of the effect on PK and PD.

Toxicology studies incorporated PK, toxicokinetic (TK), and PD analyses and were conducted in normal CD-1 mice, Sprague Dawley rats, New Zealand White (NZW) rabbits, and cynomolgus monkeys. Toxicology studies utilized both the IV and SC routes of administration to mimic the intended clinical use, to characterize the toxicity of pegzilarginase. All species utilized (mice, rats, rabbits, and cynomolgus monkeys) had normal levels of arginine, which enabled PK and PD assessment of pegzilarginase. However, since the normal arginine levels do not mimic the arginine levels in the ARG1-D disease state, there is an expected difference between the doses that drove PD-induced toxicology findings in the nonclinical evaluation compared to what may be expected clinically.

2.5.2. Pharmacology

2.5.2.1. Primary pharmacodynamic studies

Characterisation of the primary pharmacology of pegzilarginase was based primarily on *in vivo* studies. No *in vitro* data on enzymatic activity, substrate kinetics, species specificity or *in vitro* dose-response effects were submitted.

In vivo data following IV or SC administration of pegzilarginase across a wide dose range in both naïve and disease models, both rodent and non-rodent, were generated. However, it should be noted that the primary PD effects established in naïve animals are based on (pivotal) toxicology studies and are discussed in more detail. Briefly, profound and dose-dependent depletion of arginine was observed after treatment with arginase after weekly IV doses in mice, juvenile rats, adult rats, rabbits and monkeys after subacute (4 weeks) up to chronic (6 months) exposure. During the recovery period, recovery of arginine levels was not or partially achieved in the higher dose groups. Comparisons between IV and SC dosing of pegzilarginase showed comparable, dose-dependent, but delayed reductions of arginine. The PD profile after SC administration was flatter as compared to IV administration, with a smaller maximal reduction in arginine levels. Maximal reduction of arginine coincided with a SC T_{max} of 8 to 24 hours, which was delayed with respect to IV administration. Arginine return to baseline was generally completed or nearly completed, by 168 hours post-dose.

Modulation of hyperarginaemia was evaluated in three animal models of the disease. Pegzilarginase was administered via intraperitoneal injection in all three studies.

The neonatal mouse arginase 1 deficiency model (Arg1-/-) shows excess circulating arginine and catabolites of arginine. Survival in these animals is low (typically up to 14 days) as a result of model-specific hyperammonaemia. Single-dose studies with this model demonstrated that 0.04 mg/kg pegzilarginase normalised serum arginine levels in these mice. A subsequent 0,05 mg/kg dose showed

a sustained effect could be achieved for up to 72 hours. Following this, Arg1-/- animals received three doses of pegzilarginase starting on PND6 over the next five days. Arginine was reduced to levels comparable to wt-mice, corresponding to increased ornithine levels, showing effective arginine metabolism. However, a subsequent study did not result in an increase in survival, likely due to hyperammonaemia being insufficiently controlled.

In adult tamoxifen inducible arginase 1 deficiency mouse models, single doses of 0.005 to 0.04 mg/kg pegzilarginase dose dependently reduced serum arginine levels, correlated to increased plasma ornithine. An optimal dose of 0.02 mg/kg was selected for subsequent studies where animals received 2 pegzilarginase doses on day 15 and 17 after tamoxifen treatment. Similar to the juvenile study, pegzilarginase lowered serum arginine to levels comparable to wt-animals but did not improve survival as a result of hyperammonaemia.

Arginine (1 g/kg/day) was administered to naïve Wistar rats to induce and maintain hyperargininaemia. Subsequently, the animals were administered a single dose of pegzilarginase (0.01-1 mg/kg). In all dose groups, a sustained decrease in serum arginine was achieved, corresponding to increases in ornithine. Doses up to 0.03 mg/kg resulted in reductions comparable to unconditioned animals, and doses above that resulted in a more profound depletion of arginine. Based on the results from the toxicology studies that used the refined LC-MS/MS assay and the bridging PD study, it emerged that at doses higher than the NOAEL, a sustained decrease in arginine levels below the normal animal range was observed for longer than 120 hours (repeatedly weekly) in all species, including juvenile male rats, which correlated with some toxicological effects.

Effective control over serum arginine levels has been adequately demonstrated, suggesting that toxic processes driven by excess arginine or its derivatives can be limited. However, none of the studies evaluated whether this is the case and there has been no exploration of, for example, neuroprotection as a result of pegzilarginase administration.

Overall, the mode of action of pegzilarginase is relatively well understood as a function of arginase enzymatic activity. The PEG moiety extends the half-life of the molecule allowing for prolonged pharmacological action. However, a more detailed in vitro characterisation and direct comparison with rhArginase would improve the understanding of the primary PD. The proof of concept of pegzilarginase to dose-dependently reduce and deplete serum arginine in transgenic juvenile and adult disease models and an induced naïve model has been adequately demonstrated. However, whether effective control over serum arginine levels also results in clinically relevant effects, such as prevention of neurotoxicity, has not been evaluated; thus, this correlation should be demonstrated in the clinic. No secondary pharmacology studies have been conducted.

2.5.2.2. Secondary pharmacodynamic studies

No secondary pharmacology studies were conducted, which is acceptable. Since pegzilarginase is an enzyme produced via biotechnological processes, this is acknowledged in line with ICH S6.

2.5.2.3. Safety pharmacology programme

Dedicated safety pharmacology studies are not required for biotechnology products. The safety pharmacology of pegzilarginase has been evaluated in pivotal repeat-dose toxicity studies with cynomolgus monkeys and juvenile rats.

There were no toxicologically meaningful findings on the cardiovascular, CNS or respiratory systems that would suggest an acute safety concern for these vital systems based on the pharmacology of pegzilarginase.

2.5.2.4. Pharmacodynamic drug interactions

No pharmacodynamic drug interaction studies were conducted, which is agreed as it aligns with ICH S6(R1).

2.5.3. Pharmacokinetics

Methods of analysis

PK and TK were measured using enzymatic activity assays. For the measurement of arginine levels in acidified rat, mouse and monkey plasma, the LC-MS/MS methods were developed and adequately validated over the range of 1-200 µM (studies MV-2014-931, MV-2014-932, MV-2014-937 and TSLS15-107). Plasma acidification was necessary to stop the arginase activity. Due to the presence of endogenous arginine and ornithine in plasma, the calibration curve samples were prepared in a surrogate matrix, whereas the QC samples were prepared both in the surrogate matrix and the acidified plasma.

To improve the certainty of arginine measurements, study MV-2014-937 was extended with the development and validation of the analytical methods for the measurement of arginine in rat plasma stabilized with the arginase inhibitor nor-NOHA (referred to as "stabilized plasma") and for rat plasma pre-treated with pegzilarginase and subsequently stabilized with nor-NOHA (referred to a "treated plasma"). For rabbit plasma, the analytical LC-MS/MS method was developed for stabilized plasma (with nor-NOHA addition) and validated over the range 1-200 µM (study MV-2018-1079).

Based on the regulatory feedback, the analytical methods were subsequently extended to and validated for acidified rat and monkey plasma to include the measurement of ornithine (study MV-2016-1027 in rat plasma, range 1-200 µM for both substances and MV-2016-1030 in monkey plasma over the range 1-200 µM for arginine and 2-400 µM for ornithine). Additionally, to improve the certainty of arginine and ornithine measurements, the study MV-2016-1030 was extended to include the development and validation of the method to measure arginine and ornithine in monkey plasma stabilized with nor-NOHA.

For assessing the levels of the active substance pegzilarginase, the analytical methods were developed and adequately validated for acidified rat, mouse, rabbit and monkey sera based on measuring the enzymatic activity in converting radiolabelled arginine to ornithine. Briefly, following incubation in 10 mM radiolabelled arginine, the reaction was stopped by the addition of acid, the proteins were precipitated, and the supernatants evaporated to dryness, reconstituted, derivatized with hydrochloric acid in butanol and analysed for the content of radiolabelled ornithine with LC-MS/MS. As a result, the assays were adequately validated for the range of 0.125-15 µg/mL in monkey serum (study MV-2014-938) and 0.500-15.0 µg/mL in mouse (study MV-2014-939), rat (study ME-2015-948) and rabbit (study MV-2017-1072) sera. In addition, a partial validation was performed for the measurement of pegzilarginase in the treated (arginase-deactivated) rat serum (study TSLS15-108). The levels of endogenous arginase in individual animal lots and haemolyzed serum were analysed and determined to be significantly below LLOQ in monkey and mouse sera (up to 0.898% of the LLOQ in individual lots, up to 2.89% in haemolyzed serum; mouse: not detected). For rabbit and rat sera, no significant matrix interference and analyte response was observed in the individual and pooled matrix blank samples and in haemolyzed blank samples.

The validation of the above-mentioned developed methods was adequate regarding calibration, accuracy, precision, matrix effect and dilution integrity. Stability in a matrix at room temperature and long-term storage stability is considered to be sufficiently covered for all validated methods. Incurred

sample reanalyses (ISR) were conducted in pivotal toxicological studies with each test species and were within the established acceptance criteria.

The impact of ADAs on TK was analysed using developed ADA methods. ADA samples were analysed in the 6-month rat and the 13-week monkey studies as well as the 4-week IV/SC monkey toxicology studies. For ADA measurements in rat and monkey sera, the Meso Scale Discovery electro-chemiluminescent methods were developed and validated, which used biotinylated pegzilarginase (B-pegzilarginase) to capture ADAs and Ruthenium-labelled pegzilarginase (Ru-pegzilarginase) to detect the antibodies. QC samples representing a high (HPC), mid (MPC) and low (LPC) concentration of antibody were prepared by spiking pooled normal cynomolgus monkey serum with the surrogate-positive control at 2000 ng/mL (HPC), 200 ng/mL (MPC), and 40 ng/mL (LPC). A negative control (NC), consisting of pooled normal cynomolgus monkey serum, was also analyzed on all assays. The studies were conducted under the GLP compliance. Validation of the method included establishment of screening, confirmatory and titration cut points and assessment of specificity, assay sensitivity, selectivity/matrix interference, drug tolerance, prozone (hook) effect, titration assay linearity, intra- and inter-assay precision, short-term stability, freeze/thaw stability, and robustness. The drug tolerance evaluation demonstrated that the assays were able to detect surrogate positive control (SPC) at 2000 and 200 ng/mL in the presence of up to 150 µg/mL of free pegzilarginase and SPC at 40 ng/mL in the presence of up to 75 µg/mL of free perzilarginase in pooled normal cynomolgus monkey serum and SPC at 2000, 200 and 40 ng/mL in the presence of up to 150 µg/mL of free pegzilarginase in pooled normal rat serum.

Absorption

Single dose studies

The PK parameters were evaluated after a single dose administration of pegzilarginase by IV route in CD-1 mice and by IV and SC route in male Cynomolgus monkeys.

Following the IV administration in mice, the $AUC_{0-\infty}$ appeared to increase slightly less than the dose proportionately at the two top dose levels (2 and 6 mg/kg). The mean volume of distribution (Vd_{ss}) was within 1- to 1.6-fold of mouse serum volume (~50 mL/kg). Mean CL values were 0.02 and 0.025 mg/min/kg, respectively, at 2 and 6 mg/kg.

Following the IV administration in monkeys, the $AUC_{0-\infty}$ tended to increase more than dose-proportionately (up to x1.8; 30-fold increase over 16.7-fold increasing dose). When normalizedised to the dose level, the mean $dnAUC_{0-\infty}$ ($AUC_{0-\infty}/D$) increased with escalating dosing. This was consistent with decreasing CL values, diminishing from 1.20 mL/hr/kg at the dose level of 0.03 mg/kg to 0.657 mL/h/kg at the dose level of 0.5 mg/kg, suggesting that greater than proportional increases in AUCs were due to a decrease in clearance as the dose was increased. The observed volume of distribution at steady state (Vd_{ss}) was similar to the monkey serum volume (45 mL/kg). The decreasing CL resulted in the mean half-life ($T_{1/2}$) values increasing with escalating dose. Mean $T_{1/2}$ (±SD) varied in the range of 20-56 hr over the dose range of 0.03-0.5 mg/kg.

As pegzilarginase is intended to be administered both intravenously and subcutaneously, two studies in monkeys compared single IV or SC doses of pegzilarginase. The second of the two studies evaluated the single-dose PK of 2 alternative pegzilarginase formulations for SC administration. Since no improvement in PK profile was seen with these 2 new formulations, the formulation used in the first study and the previous IV study was selected for the following GLP study (and the clinic). The bioavailability of pegzilarginase by SC route was ca. 52-62%.

Repeated dose studies

The PK parameters after repeated IV administration of pegzilarginase over the range of 0.1-6 mg/kg were studied in the repeated dose toxicity studies with juvenile rats (dosing from PND 21), mice and monkeys; fertility, pre-and post-natal developmental toxicity studies with rats; developmental toxicity studies with rabbits, and in juvenile rats, using QW or once every five days (pregnant rats) dosing regimen. The SC administration route was also studied in the 4-week study with monkeys. The pivotal studies were GLP-compliant.

No consistent sex differences were observed in any species. There was a general trend of decreasing clearance with increasing dose levels on the 1st day of administration, resulting in slightly more than dose-proportionate increases of AUC0-∞, with this effect being more pronounced in rodents and rabbits and less obvious in monkeys. After steady-state achievement, the exposure increases were generally close to dose-proportionate. Steady-state was considered to be reached by Day 22 in mice, Day 36 in rats, GD20 in rabbits, and Day 22 in monkeys based on overlapping of pre-dose and terminal trough levels and sampling time. The steady-state clearance values tended to be lower compared to Day 1 in rodents and rabbits. In the prolonged administration rat studies, consistently higher (ca. 3-fold) exposure levels were seen after the achievement of steady-state compared to Day 1, indicating accumulation. However, the apparent increases in pegzilarginase concentrations and exposure with repeat dosing may have been the result of decreasing blood (and serum) volumes as the juvenile rats matured into adults while on study. This was not seen in monkeys, where the steady-state exposures were generally comparable to Day 1 values. The volume of distribution was, in general, within 2-fold of the serum volume in rats, rabbits and monkeys and notably higher in mice, particularly at the low dose level of 0.6 mg/kg (up to 191 mL/kg, compared to the serum volume of 50 mL/kg); however, the results could have been influenced by unexpected and significant inter-animal variability seen at this dose level. The T1/2 tended to increase in higher species, from 27-34 hr in mice to 36-52 hours in the monkey.

Following SC exposure of pegzilarginase in monkeys, T_{max} was prolonged, as expected, 8 hours at 0.1 mg/kg and 24 hours at 0.5 mg/kg. The C_{max} was lower compared to the IV administration, indicating lower absorption. The bioavailability could not be determined, as AUC at a dose level of 0.1 mg/kg following SC administration was unexpectedly higher than after the IV administration (SC $AUC_{0-\infty}$ = 60.9 $\pm$ 8.83 µg x hr/mL vs IV $AUC_{0-\infty}$ = 46.0 $\pm$ 1.81 µg x hr/mL, respectively).

In a GLP-compliant 4-week study with male mice (AER-MPI-006/2283-006), Cmax and AUC0-t tended to increase slightly more than dose-proportionately with increasing dose, while both Vdss and CL decreased, with Vdss being generally larger than the mouse serum volume (~50 mL/kg), especially at the low dose (155-191 mL/kg). This might suggest the saturation of target-mediated clearance at high dose levels; however, there was also significant and unexpected inter-animal variability observed in the study, with approximately 10- to 60-fold differences in pegzilarginase concentrations within certain time points/days/dose groups in particular in the low-dose group. Therefore, the low dose concentration profile did not appear to be consistent with IV administration, which may have skewed the results. As immunogenicity samples were collected only in separate animals, it was not possible to assess the possible ADA influence on the exposure. The T1/2 was independent of dose and varied from 27.4 to 34.1 hr, with comparable results on Day 1 and Day 22.

Similarly, a more than dose-proportionate increase in C_{max} and AUC0-t with a trend for decreasing clearance with increasing dose was also seen in the GLP-compliant 6-week rat study (AER-MPI-011/2283/010) and the GLP-compliant 13-week/6-month rat study (AEB-002-1020 / 2461-004) on the first day of dosing. After the achievement of steady-state, the increases in exposure were generally closer to dose-proportionate. Consistently higher exposure levels were measured after repeated dosing compared to Day 1 in both studies, indicating accumulation. Steady-state clearance appeared to be

slower than Day 1 (0.825-0.936 mL/h/kg vs 1.22-1.90 mL/h/kg in the 6-week study, 0.619-0.88 mL/h/kg vs 1.46-2.27 mL/h/kg in the 13-week/6-month study). The volume of distribution on Day 1 was approximately two-fold of the rat serum volume in both studies, while the Vdss was somewhat lower, within 1.5-fold of the rat serum volume in the 6-week study and roughly comparable to the serum volume in the 13-week/6-month study. Mean steady-state T1/2 values were overall comparable and ranged from 32 to 39 hours in the 6-week study and from 25 to 39 hours in the 13-week/6-month study.

The trend of decreasing clearance with increased dosing was also evident in the GLP-compliant studies with pregnant rats (AEB-002-1166/2461-012) and rabbits (AEB-002-1168/2461-013). The Cmax and AUC increased slightly more than dose-proportionate on both time points (GD6 and GD16/GD20 in rats/rabbits), being roughly comparable at both time points without notable accumulation. Consequently, there was also a trend to the increasing T1/2 with increased dosing in both species (rat: 19.3-25.5 hr at 0.1 mg/kg vs 31.0-34.7 at 1 mg/kg; rabbits: 16.6-18.4 hr at 0.06 mg/kg vs 26.5-35.0 hr at 0.3 mg/kg). The volume of distribution was within 1.7-fold of the rat serum volume at both time points, while in rabbits, it was slightly lower or comparable to the rabbit serum volume (44 mL/kg).

In the GLP-compliant PPND study with rats, the pup exposure was below the quantification limit at both PND 4 and PND 20.

In the 4- and 13-week studies with cynomolgus monkeys (AER-MPI-005/2283-003 and AEB-002-1014/2461-003), the exposure increased generally close to dose-proportionate both at Day 1 and at the end of the exposure. The Cmax and AUC in steady state were comparable to the respective values on Day 1, indicating no notable accumulation. In the 4-week, there was a general trend for slightly higher mean CL values after the achievement of steady-state compared to Day 1; however, this was not evident in the 13-week study. Mean steady-state volume of distribution was generally similar to monkey serum volume (45 mL/kg). Across all animals, T1/2 ss ranged from 35.9 to 48.6 hr in the 4-week study and from 41.0 to 50.4 hours in the 13-week study.

Following subcutaneous administration in monkeys (AEB-002-1152 / 2461-008), exposure increased more than dose-proportionate on Day 1 and roughly dose-proportionate on Day 22. There was a slight trend on Day 1 for decreasing mean extravascular clearance (CL/F) with increasing dose, consistent with the apparent greater-than-dose proportional increases in AUC0-∞. On day 22 mean CLss/F was relatively constant on both tested dose levels, with mean T1/2 ranging from 48.5 $\pm$ 2.91 to 46.3 $\pm$ 3.55 hr. No notable accumulation was seen. Mean Vz/F was within 2.1-fold of monkey serum volume on both time points.

Distribution

No distribution studies have been conducted by the applicant. The volume of distribution following single or repeated administration was, in general, within 2-fold of the serum volume in rats, rabbits and monkeys, suggesting the distribution within the blood compartment. A higher volume of distribution was seen in mice (up to 191 mL/kg, compared to the serum volume of 50 mL/kg); however, the results might have been skewed by unexpected and significant inter-animal variability seen at the low dose level. Following the repeated administration, the steady-state Vd tended to be lower compared to Day 1.

As pegzilarginase is a pegylated enzyme intended to be used in the paediatric population (children ≥ 2 years old), the applicant has provided considerations on possible PEG-related toxicity. The applicant argues that no PEG-related safety concerns are envisaged for pegzilarginase administration in paediatric patients, as pegzilarginase utilizes 5 kDa PEG, and no vacuolation was observed at the histopathological evaluation of mice, rats and monkeys administered pegzilarginase for the period up to 6 months. While this is acknowledged, PEG-related vacuolations have also been previously observed

with smaller PEG moieties (< 40 kDa), and the current CHMP Safety Working Party Guideline on Development of PEGylated drug products for use in Paediatric Patients (2012) states that the applicant should address whether the PEGylated drug product may undergo active transport across the blood-CSF barrier and investigate the biodistribution of the PEGylated drug product, unless the monthly PEG exposure is significantly lower than the cases where ependymal cell vacuolation has been observed (≥0.4 µmol/kg/month). The assessor calculated the monthly PEG exposure by considering the worst-case molecular weight of 224 kDa for pegzilarginase and a maximal number of 14 PEG units per molecule and was found to be 0.05 µmol/kg/month. Taking into consideration the low PEG molecular weight, the lack of vacuolation in the histopathological evaluations in the repeated dose toxicity studies and the calculated monthly PEG exposure significantly below the trigger value defined in the CHMP Guideline, it was agreed that no concerns from PEG-related toxicity are envisaged for paediatric patients due to administration of pegzilarginase.

Metabolism

No metabolism studies were conducted in non-clinical species. This is agreed upon since pegzilarginase is a recombinant enzyme and is expected to be degraded similar to other enzymes. Literature data on the metabolism of pegylated enzymes suggest that the protein part is degraded by proteolysis to short peptide and amino acids by lysosomal enzymes until the PEG is cleaved from the amino acid backbone, for example, by hydrolysis of the linker moiety. As pegzilarginase utilizes a relatively small (5 kDa) PEG, the resulting PEG molecules are expected to be cleared by renal filtration.

Excretion

No dedicated excretion studies were conducted in non-clinical species. This is agreed upon since pegzilarginase is a recombinant enzyme and is expected to be eliminated similarly to other enzymes.

Pharmacokinetic drug interactions

No drug-drug studies were conducted in non-clinical species. This is agreed upon since pegzilarginase is a human recombinant enzyme.

Other pharmacokinetic studies

ADA formation was studied in the 6-month GLP-compliant rat study (AEB-002-1020 / 2461-004) and the 13-week GLP-compliant study with monkeys (AEB-002-1014 / 2461-003). No ADA formation was evidenced in any of the tested animals in the rat study (32/dose group). In monkeys, ADA formation was confirmed in 6/26 animals (2 at each tested dose level); however, this did not impact the resulting exposure.

2.5.4. Toxicology

2.5.4.1. Single dose toxicity

In a single-dose toxicity study in mice, an MTD of 6 mg/kg/day was established.

2.5.4.2. Repeat dose toxicity

Mice at 8 mg pegzilarginase/kg/week IV experienced severe toxicity within a week, probably caused by a profound and sustained depletion of serum arginine. After 4 weeks at 6 mg/kg/week, decreased food consumption and mild decreases in red cell mass with concurrent increases in reticulocytes and

platelets were seen, considered secondary to general hematopoietic stimulation, which resolved after recovery. The NOAEL was 6 mg/kg, which relates to 14 times the intended exposure in humans based on AUC. In juvenile rats (dosing initiated on PND 21), decreased food consumption and body weight loss were observed at 3 mg/kg after 6 weeks, with decreases in erythrocyte counts, which were considered adverse due to the magnitude of change. Mild decreases were seen in lymphocyte counts, eosinophils, urea nitrogen, creatinine, total protein, albumin, and globulin, which had largely resolved by the end of the recovery period. The testes showed minimal to mild bilateral degeneration/atrophy of the seminiferous tubules characterised by degeneration and loss of germinal cells, multinucleated cells, and decreased spermatids. The secondary sex glands (prostate, coagulating glands, and seminal vesicles) were decreased in size and less developed (hypoplasia). Additionally, the femur and tibia lengths were 6% and 8% lower, respectively, which was considered adverse. Notably, these changes in male reproductive organs observed at 3 mg/kg were not observed in the GLP 4-week mouse and monkey studies conducted in young adult animals rather than juveniles. After recovery, mild bilateral degeneration/atrophy of seminiferous tubules and minimal to moderate oligospermia and increased germ cell in tubules of epididymides were still present.

Changes in bone marrow and spleen were likely a regenerative response associated with decreases in peripheral red cell mass observed. Following the end of the recovery period, these microscopic findings in the bone marrow were no longer observed; however, a minimal increase in extramedullary haematopoiesis (EMH) was present. Based on the above findings, 1 mg/kg was considered the NOAEL for pegzilarginase in juvenile rats dosed for 6 weeks (7 times the human exposure). However, in the 6-month study in juvenile rats, effects on sperm worsened, and degenerative spermatids occurred, which lowered the NOAEL for males to 0.1 mg/kg/week, which is only 0.2 times the human exposure.

In adult cynomolgus monkeys, a dose of 1.75 mg/kg was given one week after a dose of 0.3 mg/kg appeared already lethal. In the 3-month study at 1 mg/kg/week, body weight decreased in such a way that animals were provided supplemental food enrichment. In addition, increased incidences of sparse hair, dry/discoloured skin, tremors, inappetence, watery faeces, decreased activity, ataxia, muscle wasting, and hunched appearance were shown, and comparable haematology changes as seen in rats. But no effects on testes were seen. The NOAEL was considered 0.3 mg/kg/week, which relates to 2 times the intended human exposure.

Importantly, there were no apparent PEGylation effects seen in all animals (i.e., cellular vacuolation of phagocytic or nonphagocytic cells related to the PEG moiety) observed by histopathology.

2.5.4.3. Genotoxicity

No genotoxicity studies have been conducted with pegzilarginase, which is acceptable. The range and type of genotoxicity studies routinely conducted for small molecule drug products are generally not applicable to biotechnology-derived products [ICH S6(R1)]. It is not expected that a biological product, such as pegzilarginase, would interact directly with deoxyribonucleic acid (DNA) or other chromosomal material.

2.5.4.4. Carcinogenicity

No conclusive evidence was observed for the role of arginase 1 as a mediator of carcinogenicity. Based on the nature of the substance (pegylated form of a human recombinant enzyme), pegzilarginase is expected to present a low carcinogenicity risk to ARG1-D patients. Furthermore, the performance of conventional carcinogenicity studies is probably not practically feasible, considering the adverse effects caused by arginine depletion. Accordingly, no standalone carcinogenicity testing studies are considered necessary.

2.5.4.5. Reproductive and developmental toxicity

In the rat fertility study, male rats showed a significant decrease in sperm motility and concentration and a significant increase in abnormal sperm morphology at 1 mg/kg. In naïve females paired with these treated males, a test article-related effect was apparent in the GD 13 pregnancies as a significant reduction in uterine implantation sites and increased pre-implantation loss were observed. At 1 mg/kg, treated females had an increase in the number of non-pregnant animals, which reduced both the fertility and fecundity indices. There were no test article-related observations in males and females treated at ⩽0.3 mg/kg/week, of which the exposure is about the same as in humans, based on AUC.

In the embryo-foetal development study in rats, females endured severe weight loss at 1 mg/kg, and offspring showed weight loss and significant increases in the incidence of bent scapula, overall skeletal malformations, incompletely ossified interparietal bone, and incompletely ossified parietal bone. These findings are probably transient secondary effects resulting from the maternal and foetal toxicities at that dose, a common manifestation of malnutrition during pregnancy. The NOAEL for maternal and developmental toxicity in rats was 0.3 mg/kg/dose.

In the embryo-foetal development study in rabbits, at 0.3 mg/kg/week, females showed a decrease in body weight and uterine weight. Foetal body weights were >20% decreased, but no adverse effect of pegzilarginase was observed on foetal external, visceral, or skeletal examinations. The NOAEL for maternal and developmental toxicity was 0.1 mg/kg/week, which is about 0.4 times the human exposure based on AUC.

In the prenatal and postnatal development study in rats, females suffered body weight loss at 1 mg/kg. No adverse effects of pegzilarginase were observed on the mean number of liveborn pups/litter at birth, the mean number of stillborn pups/litters at birth, percent of stillborn pups/litter and F1 pups detailed examination. However, at 1 mg/kg, pegzilarginase-related toxicity was observed in decreased F1 pup body weights during lactation, at the time of vaginal opening or preputial separation, and for most of the growth period overall. Also, there was an effect on learning and memory from the passive avoidance testing in males, which was considered pegzilarginase-related and adverse. Probably, because of exaggerated pharmacology due to sustained depletion of arginine below the normal range.

The NOAEL for general toxicity in parental female rats was 0.3 mg/kg, based on reduced body weight at 1 mg/kg. The NOAEL for reproductive function in parental females was 1 mg/kg. The NOAEL for F1 male and female general toxicity was 0.3 mg/kg due to the decreased mean body weights and effects on learning and memory from the passive avoidance testing (males only) at 1 mg/kg. The NOAEL for F1 male and female fertility and reproductive parameters was 1 mg/kg (about 3 times the human exposure).

2.5.4.6. Toxicokinetic data

The steady-state exposures (AUC0-∞) at the NOAEL levels in the repeated exposure animal studies were compared with the anticipated clinical exposure at week 24 at the maximum recommended dose of 0.2 mg/kg (219 mg x hr/mL), based on the results of the clinical study CAEB1102-300A. As can be seen, at the lowest NOAEL of 0.1 mg/kg, established in the 6-months study with rats, the exposure multiple is < 1.

After achieving a steady state, the exposure levels were generally close to dose-proportionate in all species. The steady-state was considered to be reached by Day 22 in mice, Day 36 in rats, GD20 in rabbits, and Day 22 in monkeys based on overlapping of pre-dose and terminal trough levels and sampling time. Steady-state values in naïve rats were higher than exposure on Day 1 (~3 fold),

indicating accumulation; this was not seen in monkeys where steady-state exposures were generally comparable to Day 1. No sex differences were observed in any species.

Anti-drug antibodies were not detected in any of the tested animals in the 6-month rat study (32/dose group). In the 13-week study with monkeys, ADA formation was confirmed in 6/26 tested animals (2 at each dose level); however, the ADA formation had no apparent impact on exposure.

2.5.4.7. Local tolerance

No findings on injection sides in the repeat-dose studies were reported in the mouse. In the cynomolgus monkey there were mild to moderate epidermal hyperplasia which resolved after recovery. Pegzilarginase was well tolerated in a SC toxicology study in the monkey, providing manageable local tolerance and the absence of PEG-related dermal toxicities.

2.5.4.8. Other toxicity studies

Since pegzilarginase is a recombinant PEGylated protein and foreign to non-human species, it is, therefore, likely to be immunogenic in them. The theoretical risk of immunogenicity to pegzilarginase was considered primarily associated to PEGylation. To support the interpretation of the PK and thus the toxicological exposure to pegzilarginase as well as determine any potential toxicological consequences, the incidence of ADAs (i.e., anti-pegzilarginase and anti-PEG antibodies) was evaluated routinely in nonclinical studies in both rats and monkeys. Development of ADAs were infrequent and did not impact exposure or impact or arginine reduction.

The haemolytic potential of pegzilarginase was evaluated *in vitro* and *in vivo* and found negative.

No indication of PEG-related toxicity, such as renal changes or vacuolation, was observed microscopically in mice, rats, or monkeys administered pegzilarginase.

2.5.5. Ecotoxicity/environmental risk assessment

The active substance is a recombinant form of a natural enzyme, the use of which will not alter the concentration or distribution of the substance in the environment. Therefore, pegzilarginase is not expected to pose a risk to the environment and therefore conducting and submitting ERA studies is not deemed necessary for such substances. Pegzilarginase is PEGylated with (mPEG), however this is not a pharmacologically active moiety and hence is not considered environmentally relevant.

2.5.6. Discussion on non-clinical aspects

Pharmacodynamics

The applicant has submitted a discussion on binding kinetics and relative potency of pegzilarginase based on literature data. Briefly, this data suggests that the substitution of Mn^{2+} for Co^{2+} results in a more potent and stable enzyme, while there are no remarkable differences in the structure or metal ion coordination. The binding site for pegzilarginase is identical in all species; therefore, no analysis of the relative binding kinetics across a dose range in other species was considered necessary.

Pharmacokinetics

Exposure to pegzilarginase was studied in the single and repeated dose toxicity studies in juvenile Sprague-Dawley rats, CD-1 mice and Cynomolgus monkeys (up to 6 months in rats, 4 weeks in mice and 13 weeks in monkeys), as well as in fertility and embryo-foetal development (FEED), embryo-foetal development (EFD) and pre-and post-natal development (PPND) studies in rats, EFD studies in New Zealand White rabbits and repeated dose toxicity studies in juvenile rats. The majority of the studies was performed by IV route, with the dose levels ranging from 0.1 to 6 mg/kg using weekly bolus injection regimen. The SC route administration was studied in 2 single-dose and 1 repeated dose (4 weeks) studies in monkeys.

The analytical methods were developed and adequately validated for the measurements of pegzilarginase substrate arginine by LC-MS/MS in acidified rat, mouse and monkey plasma over the range of 1-200 µM, for the measurements of both arginine and its conversion product ornithine by LC-MS in acidified rat and monkey plasma (validated over the range of 1-200 µM for both substances in rat plasma and 1-200 µM for arginine and 2-400 µM for ornithine in monkey plasma), and for the measurements of arginine and ornithine in rat, monkey and rabbit plasma stabilised with arginase inhibitor nor-NOHA. For assessing the levels of the active substance pegzilarginase, the analytical LC-MS/MS methods were developed and adequately validated for acidified rat, mouse, rabbit and monkey sera, based on measuring the enzymatic activity in converting radiolabelled arginine to ornithine (^{15}N2-ornithine or ^{13}C5-ornithine). The validation of the above-mentioned developed methods was adequate regarding calibration, accuracy, precision, matrix effect and dilution integrity. Stability in a matrix at room temperature and long-term storage stability were considered to be sufficiently covered for all validated methods. For ADA measurements in rat and monkey sera, the Meso Scale Discovery electro-chemiluminescent methods were developed and validated, which used biotinylated pegzilarginase (B-pegzilarginase) to capture ADAs and Ruthenium-labelled pegzilarginase (Ru-pegzilarginase). A negative control (NC), consisting of pooled normal cynomolgus monkey serum, was also analyzed on all assays. Validation of the method included establishment of screening, confirmatory and titration cut points and assessment of specificity, assay sensitivity, selectivity/matrix interference, drug tolerance, prozone (hook) effect, titration assay linearity, intra- and inter-assay precision, short-term stability, freeze/thaw stability, and robustness. The studies were conducted under GLP compliance.

In the pivotal GLP-compliant repeated dose toxicity studies with pegzilarginase, no consistent sex differences in exposure were observed in any species. There was a general trend of decreasing clearance with increasing dose levels on the 1st day of administration, resulting in slightly more than dose-proportionate increases of C_{max} and $AUC_{0-\infty}$, with this effect being more pronounced in rodents and rabbits and less obvious in monkeys. After the achievement of steady-state the increases in exposure were generally closer to dose-proportionate. A steady state was considered to be reached by Day 22 in mice, Day 36 in rats, GD20 in rabbits, and Day 22 in monkeys based on overlapping of pre-dose and terminal trough levels and sampling time. The steady-state clearance values tended to be lower compared to Day 1 in rodents and rabbits. In the prolonged administration rat studies, consistently higher exposure levels were seen after the achievement of a steady state compared to Day 1 (ca. 3-fold), indicating accumulation. However, the apparent increases in pegzilarginase concentrations and exposure with repeat dosing may have been the result of decreasing blood (and serum) volumes as the juvenile rats matured into adults while on study. This was not seen in monkeys, where the steady-state exposures were generally comparable to Day 1 values.

The bioavailability of pegzilarginase by SC route was ca. 52-62% based on a non-GLP single-dose study in male Cynomolgus monkeys. Following SC repeated dose administration of pegzilarginase to monkeys, T_{max} was higher, as expected, being 8 hours at 0.1 mg/kg and 24 hours at 0.5 mg/kg, while C_{max} was lower compared to the IV administration, indicating lower absorption.

The applicant has submitted the data which demonstrated that pegzilarginase was well tolerated by both IV and SC routes of administration with similar overall safety profiles, and both routes of administration resulted in a consistent and sustained reduction in plasma arginine and an increase in plasma ornithine levels.

No distribution studies have been conducted by the applicant. In general, the distribution was within 2-fold of the serum volume in rats, rabbits and monkeys, suggesting the distribution within the blood compartment. In mice, the volume of distribution was notably higher, particularly at the low dose level of 0.6 mg/kg (up to 191 mL/kg, compared to the serum volume of 50 mL/kg); however, the results could have been influenced by unexpected and significant inter-animal variability seen at this dose level. Following the repeated administration, the steady-state Vd tended to be lower compared to Day 1.

As pegzilarginase is a pegylated enzyme intended to be used in the paediatric population (children $\geqslant$ 2 years old), the applicant has provided considerations on eventual PEG-related toxicity. Taking into consideration the low PEG molecular weight, the lack of vacuolation in the histopathological evaluations in the repeated dose toxicity studies and the calculated monthly PEG exposure being significantly below the trigger value defined in the CHMP Safety Working Party Guideline on Development of PEGylated drug products for use in Paediatric Patients (2012) (0.05 vs 0.4 µmol/kg/month), it was agreed that no concerns from PEG-related toxicity are envisaged for paediatric patients due to administration of pegzilarginase.

No metabolism studies were conducted in non-clinical species. This is agreed upon since pegzilarginase is a recombinant enzyme and is expected to be degraded similar to other enzymes.

No dedicated excretion studies were conducted in non-clinical species. This is agreed upon, since pegzilarginase is a recombinant enzyme and is expected to be eliminated similar to other enzymes. Kinetic studies in non-clinical species indicated that the elimination half-life tends to increase from lower to higher species, ranging from 27 to 34 hours in mice, 18 to 41 hours in rats, 16 to 35 hours in rabbits, and 37 to 52 hours in the monkey. In rodents and rabbits, there was a tendency to decreasing clearance with increasing dose levels and a lower clearance at steady state following repeated administration compared to a single administration. The steady-state clearance varied in the range of 1.8-3.0 mL/h/kg in mice, 0.6-0.9 mL/h/kg in rats, 0.8-1.4 mL/h/kg in rabbits and 0.6-1.0 mL/h/kg in monkeys.

Toxicology

Most or all effects were likely due to exaggerated pharmacology in normal animals with normal circulating arginine levels. The applicant has provided an explanation that the contrasting effect on sperm seen between rats and monkeys could be due to the difference between the age of the animals (level of pubertal development) between the two species at the start of dosing. A substantial number of monkeys were also exposed to pegzilarginase and thus experienced hypoarginaemia during a key period of testicular development, which was further confirmed in the 13-week monkey study. Thus, effects on matured sperm have not been determined in monkeys, and a similar detrimental effect in monkeys as in rats cannot be ruled out.

In these models, the hypoarginaemia effect makes interpretation of the toxicity difficult, and the SmPC 5.3 text also reflects this fact.

Besides, substituting the native manganese (Mn^{2+}) with cobalt (Co^{2+}) in the active site of arginase 1 enhances stability and catalytic activity and may theoretically cause side effects. The total amount of cobalt to which a person receiving pegzilarginase treatment might be exposed was calculated, and it was demonstrated that the exposure remains within the permitted daily expose (PDE) of 5 µg/day set for Co by parenteral route according to ICH Q3D (R1) guideline. Additionally, substituting manganese

(Mn2+) with cobalt (Co2+) in the active site of human arginase 1 does not change the enzyme's structure. However, it increases its specificity for the preferred substrate, arginine, making it even more unlikely that an alternative substrate outside of arginine could be metabolised by pegzilarginase. Based on this information, it was agreed that the substitution of Mn^{2+} with Co^{2+} is not expected to result in side effects.

As with the repeat dose toxicity studies, the adverse effects seen in the reproductive and developmental toxicity studies were probably caused by exaggerated pharmacology due to sustained depletion of arginine below the normal range. No indication of PEG-related toxicity, such as renal changes or vacuolation, was observed in the animals. This is in agreement with the very low exposure to PEG of 0.14 mg PEG/kg/mth = 0.03 μmol PEG/kg/month, instead of $\geq$ 0.4 μmol PEG/kg/month and the low molecular mass of 5 kDa instead of 40 kDa of the used type of PEG (CHMP Safety Working Party's response to the PDCO regarding the use of PEGylated drug products in the paediatric population, EMA/CHMP/SWP/647258/2012).

Although pegzilarginase is a pegylated recombinant enzyme, no concerns for the paediatric population from eventual PEG exposure are anticipated, based on the low molecular weight (5 kDa) of the PEG units, the lack of vacuolation in the long-term rodent and monkey studies, and the estimated PEG exposure being significantly below the trigger value of 0.4 µmol/kg/month (0.05 µmol/kg/month). Long-term risks from PEG exposure are thus not envisaged.

Pegzilarginase $PEC_{surfacewater}$ value is below the action limit of 0.01 µg/L. and is not a PBT substance as log K_{ow} does not exceed 4.5.

Therefore, pegzilarginase is not expected to pose a risk to the environment.

2.5.7. Conclusion on the non-clinical aspects

Loargys is considered approvable from a non-clinical point of view.

2.6. Clinical aspects

2.6.1. Introduction

GCP aspects

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

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

Table 1 Clinical development programme

Study Number (Status)	Study Design	Population, number of subjects	Pegzilarginase Dosage/Regimen	Primary and secondary Endpoints
CAEB1102-101A (Completed)	Phase 1/2, open-label study	Paediatric and adult subjects with ARG1-, N=16	Part 1 (single ascending dose): 0.015, 0.03, 0.06, 0.10, and 0.20 mg/kg IV Part 2 (repeat dose): 0.015, 0.03, 0.04, 0.06, 0.09, 0.12, or 0.2 mg/kg IV for up to 8 QW doses	**Primary:** safety and tolerability **Clinical pharmacology:** Plasma arginine/ ornithine, GCs, PK, ADAs
CAEB1102-102A (Ongoing)	Long-term safety (open-label extension study)	Paediatric and adult subjects with ARG1-D from Study CAEB1102-101A, N=14	Continued dosing for up to 3 years. Option of SC dosing after at least 24 weeks of IV dosing	**Primary:** safety and tolerability **Clinical pharmacology:** Plasma arginine ornithine, GCs PK, ADAs
CAEB1102-300A (Double-blind period completed; Open-label LTE ongoing)	Phase 3, randomised, double-blind, placebo-controlled	Paediatric and adult subjects with ARG1-D, N=32	0.05 to 0.2 mg/kg QW IV (double-blind) or SC (LTE)	**Primary**: efficacy Based on plasma arginine **Clinical pharmacology:** ornithine, GCs PK, ADAs

Abbreviations: ADA=anti-drug antibody; ARG1-D=arginase 1 deficiency; GCs=guanidino compounds, IV=intravenous; LTE=long-term extension; PK=pharmacokinetics; QW=once weekly.

The main study (CAEB1102-300A) consisted of a 24-week randomised placebo-controlled phase (completed), followed by a long-term extension phase where all subjects were eligible for pegzilarginase.

2.6.2. Clinical pharmacology

2.6.2.1. Pharmacokinetics

The pharmacokinetics (PK) of pegzilarginase have been investigated in paediatric and adult subjects with ARG1-D in three studies, Phase 1/2 clinical study CAEB1102-**101A,** open-label extension study CAEB1102-**102A**, and pivotal Phase 3 clinical study CAEB1102-**300A**. No healthy volunteer studies were conducted.

PK data on IV administration are available for a total of 37 patients, 16 patients from study **101A** and long-term extension study **102A**, and 21 subjects from study **300A**, assigned to pegzilarginase in the double-blind period; no PK data were collected in the long-term extension part of study **300A**.

PK concentration data and parameters following QW SC administration are available for 13 subjects enrolled into extension Study 102A; one subject was discontinued prior to the switch to SC dosing. In

study **300A**, no PK data were collected following SC dosing, although patients could switch to SC dosing.

In study 102A, the median t_{max} generally occurred at, or just after, the end of IV infusion (≤2.2 hours) and between 24 and 48 hours following SC administration. Pegzilarginase C_{max} was lower following SC administration compared to corresponding dose levels after IV infusion, with absolute bioavailability (F) ranging from 33.1% to 75.8%. The mean $t_{1/2}$ was approximately 44 hours (range: 38 to 56 hours) following IV administration and 63.8 hours (range: 34.3 to 98.9 hours) following SC administration. No differences were observed across doses.

The SC and IV routes of administration were also compared using the population PK model. The PopPK included a depot compartment from which pegzilarginase was absorbed into the systemic circulation. The PopPK model-estimated F following SC dosing was 58%. The 168-hour post-dose pegzilarginase concentration was similar between IV and SC dosing.

Methods

Bioanalytical methods

Validated bioanalytical methods were used to support the clinical development of pegzilarginase. During drug development process following validation methods were developed: determination of AEB1102 activity in human serum using LC-MS/MS, determination of pegzilarginase (AEB1102) activity in human serum using LC-MS/MS (low range), the determination of AEB1102 (Co-ArgI-PEG) enzymatic activity in converting labelled arginine to labelled ornithine in human serum by LC-MS/MS, determination of arginine and ornithine in human plasma by uHPLC/MS/MS, analysis of a-K-d-GVA, (R,S)-ArgA, homoarginine HCl, and N-a-Acetyl-L-arginine in K2EDTA human plasma by LC-MS/MS, direct binding ELISA for the detection of antibodies against PEG in human serum, and determination of the relative titer of anti-PEG IgG antibodies in human serum using an electrochemiluminescent method. Generally, the strategy for development of bioanalytical methods is acceptable and methods were performed according to the EMA recommendations.

- Bioanalysis of pegzilarginase

Enzyme activity assays with an LC-MS/MS endpoint have been used to determine the concentration of pegzilarginase in human serum samples. Over time different assay ranges have been used, and different laboratories have been involved. In the clinical programme, the applicant used an indirect method of pegzilarginase level determination based on the measured activity of the enzyme. Direct methods were considered but rejected due to various reasons. As the selected indirect methods allow accurate determination of the pegzilarginase level, using indirect methods is considered acceptable.

- Within-study performance has been validated in all clinical studies. The data on incurred sample reanalysis were not completely consistent between the study reports and the submitted overview. Based on study reports, >10% of the study samples were reanalysed, and > 90% were within ±20% of the original result.
- Bioanalysis of arginine and ornithine

In the clinical studies, a UHPLC-MS/MS assay with protein precipitation has been used for the determination of arginine and ornithine in human plasma. Plasma samples for the determination of arginine and ornithine were collected in fit-for-purpose tubes containing the arginase inhibitor Nω-hydroxy-nor-arginine (nor-NOHA) to prevent *ex vivo* reduction of arginine levels with pegzilarginase.

Plasma samples were subsequently acidified to stabilize arginine to freeze-thaw cycles further and prevent further arginine metabolism with pegzilarginase.

Method performance was generally appropriate with accuracy within 20% and precision within the 15% limits; this is sufficient for a PD parameter.

- Bioanalysis of guanidino compounds (GCs)

The concentrations of four guanidino compounds (arginine-derived metabolites) were determined as secondary PD parameters. UHPLC-MS/MS assays with protein precipitation have been used for the determination of guanidino compounds (α-k-δ-guanidinovaleric acid (GVA), argininic acid (ArgA), Nα-acetyl-L-arginine (NAArg), L-homoarginine hydrochloride (HArg) and guanidinoacetic acid (GAA) in human plasma.

The bioanalysis of HArg was stopped because the HArg assay failed analytical runs for various reasons. As results for the other arginine-derived metabolites that could be measured were in line with results for the primary PD parameter arginine, and the role of HArg compared to the other guanidino compounds is not fully understood, the lack of data on HArg is accepted.

- Bioanalysis of anti-drug antibodies (ADAs)

Appropriate Anti-drug antibody (ADA) assays have been used to assess the immunogenicity risks of pegzilarginase. In the clinical studies, ADAs determination was performed by validated assays and method validation reports were provided. Evaluation and characterisation of anti-drug antibody (ADA) development followed a 3-tiered approach (screening, confirmation, and titer) using a bridging electrochemiluminescence (ECL) immunoassay method to detect ADA against pegzilarginase and a direct binding assay to detect ADA against PEG. Validation BAEU-20-116-136 Long-term stability results were not obtained during validation, and a justification was provided based upon literature data (Harlow and Lane (1988), Michaut et al. (2014) and Pihl et al. (2014)) and through industry practice. The provided justification is considered acceptable.

Evaluation and characterisation of ADA development followed a 3-tiered approach. The applicant attempted to develop an assay to assess neutralising anti-drug antibody (NAb) activity. However, despite repeated and extensive efforts, the sponsor was unable to generate a suitable positive control for the development and validation of a NAb assay. Instead, an alternative method was used. The applicant conducted an integrated pharmacokinetic (PK)-pharmacodynamic (PD)-ADA analysis to evaluate the impact of ADA on efficacy as measured by the magnitude of reduction in plasma arginine levels. This approach is considered acceptable.

In subjects who experienced a hypersensitivity reaction, complement C3, tryptase, and IgE samples were taken 3 and 24 hours post-event to evaluate the aetiology of the reaction. Further, in study **300A** IgM antiPEG antibodies and IgG antiPEG antibodies were assayed in subjects with a hypersensitivity reaction.

Pharmacokinetic data analysis

All PK parameters from the 3 clinical studies were calculated using conventional non-compartmental analysis (NCA) methods. Standard PK variables were analysed. Furthermore, all serum concentration data were analysed using population PK analysis.

Population PK and PKPD Models for Pegzilarginase

The applicant developed a population pharmacokinetic (PK) and pharmacokinetic-pharmacodynamic (PKPD) model for pegzilarginase following intravenous (IV) and subcutaneous (SC) dosing in order to: to understand the exposure of the drug and its relationship to drug effect (reduction in L-arginine),

understand the influence of patient characteristics, and to quantify the between and within patient variability.

- **Population PK Model (AEB1102) (Study 265402)**

A PopPK model has been developed to describe the PK of pegzilarginase following IV and SC dosing and to understand the influence of covariates such as age, body weight and ADAs on the PK of pegzilarginase. The final PK(PD) dataset included 37 subjects with arginase-1 deficiency, with a total of 1258 PK observations in the nonlinear mixed effects modelling software (NONMEM) dataset for model building. Almost all post-dose BLQ observations were from study CAEB1102-101A, where the LLOQ was relatively higher (0.25 µg/mL) compared to the other two studies (0.02 µg/mL). Subjects (17 male, 20 female) were aged between 2 – 31 years (6 subjects > 18 yrs), with baseline body weights ranging from 12.2 – 76.7 kg. Both data following IV and SC dosing were included.

The final model was a 2-compartment disposition and first-order elimination model, with first-order absorption for SC dosing (Table 2). Disposition of pegzilarginase was modelled for clearance (CL), the central volume of distribution (Vc), intercompartmental clearance (Q), the peripheral volume of distribution (Vp) with a first-order absorption rate constant (Ka), and bioavailability (F) for SC dosing. Between-subject variability (BSV) was included on CL, Vc, F, and Ka, with a correlation term included for the BSV terms between CL and Vc.

Table 2 Parameter estimates for the final pegzilarginase population PK model

Parameter Name	Estimated Value (%RSE)
Clearance (CL, L/h)	0.029 (2.8)
Covariate of Anti-PEG ADA titer on CL	2.56 (7.0)
Intercompartmental Clearance (Q, L/h)	0.014 (30.5)
Covariate of Weight on CL and Q	0.75 FIX
Central Volume of Distribution (V_c, L)	1.33 (2.6)
Peripheral Volume of Distribution (V_p, L)	0.438 (10.2)
Covariate of Weight on V_c and V_p	0.717 (5.4)
Rate of Absorption for SC administration (k_a, 1/h)	0.0286 (7.2)
Bioavailability for SC administration (F, fraction)	0.582 (7.3)
Between-Subject Variability for CL (CV%)	12.6 (11.5)
Between-Subject Variability for V_c (CV%)	13.8 (14.8)
Correlation CL-V_c (-)	0.668 (15.3)
Between-Subject Variability for F (additive[a])	0.493 (26.5)
Between-Subject Variability for k_a (CV%)	26 (22.8)
Residual Unexplained Variability (CV%)	25.2 (6.2)

Abbreviations: ADA=anti-drug antibody; IV=intravenous; PEG=polyethylene glycol; RSE=relative standard error; SC=subcutaneous.

Notes: $CL=0.029\times(Weight/31)^{0.75}\times(1+((anti\text{-}PEG\ titer)/100)^{2.56})$

$Q=0.014\times(Weight/31)^{0.75}$

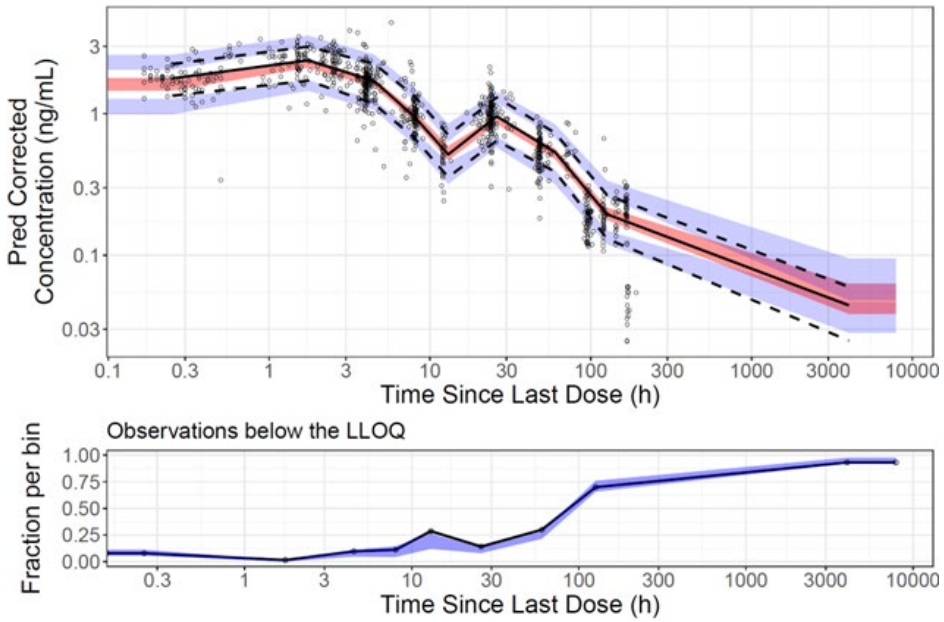
$V_c=1.33\times(Weight)/31)^{0.717}$

$V_p=0.438\times(Weight/31)^{0.717}$

[a]→additive variance on a logit scale

The population-PK model was evaluated using graphical representations of goodness-of-fit, and prediction-corrected VPCs were constructed (Figure 3). In addition, further model-derived PK parameters following IV and SC administration were compared to NCA results from studies CAEB1102-300A (IV dosing) and CAEB1102-102A (SC dosing).

Figure 3 Prediction-corrected VPC for the two-compartment pegzilarginase population PK model



Weight and anti-PEG ADA effect (titer) were significant covariates for some PK parameters and were included in the model. Anti-pegzilarginase ADAs also affected PK, but the effect of anti-PEG ADAs was larger, so anti-PEG ADAs were included in the model.

Weight was included as a significant covariate on CL, Vc, Q, and Vp, it was scaled allometrically (not estimated) on CL and Q, and a weight effect was estimated on Vc and Vp. Each of these parameters increased with increasing body weight. However, body weight had minimal impact on the exposure of pegzilarginase because of the weight-based dosing strategy. Further, anti-PEG ADA effect (titer) was included on CL. The impact of the covariates on C_{max} at steady state was minor and not clinically meaningful.

After the inclusion of weight effects in the model, no apparent trends remained for any potential effects of age and sex. Further, race, anti-pegzilarginase ADAs, CrCL, ALT, AST, ALB, and bilirubin were explored graphically and/or tested in the Pop PK model and not found to be statistically significant.

The pharmacokinetics of pegzilarginase was best described with the final 2-compartment PK model without BLQ samples. The applicant also tested the 2-compartment model using the M3 method to account for the high number of BLQ observations. This resulted in a high condition number and low precision of parameters; therefore, it was decided to exclude all BLQ observations and not to use the M3 method. It was also evaluated whether a one-compartment model with the M3 method could improve the model performance, but no improvement was observed.

- **Population PKPD model (AEB1102) (Study 265402)**

A PopPKPD Model has been developed to:

a) Describe the L-arginine profile following IV and SC dosing of pegzilarginase,

b) Understand the influence of covariates on the L-arginine profile,

c) Calculate the observed time that L-arginine was <40 µM, 40-115 µM, 40-200 µM, and <200 µM (guideline recommended level) for each study, route of administration and dose,

d) Understand the relationship between PK area under the plasma concentration-time curve (AUC) and time in ranges for L-arginine listed above for each study, route of administration and dose, and

e) Understand the impact of one and two missed doses on the L-arginine profile

using a pharmacokinetic-pharmacodynamic (PKPD) model with data from patients with arginase 1 deficiency. There was a total of 3166 plasma arginine observations in the NONMEM dataset, with 892 excluded during model building. Most of the excluded observations were because they were from the placebo treatment group in Study CAEB1102-300A.

The following assumptions have been made:

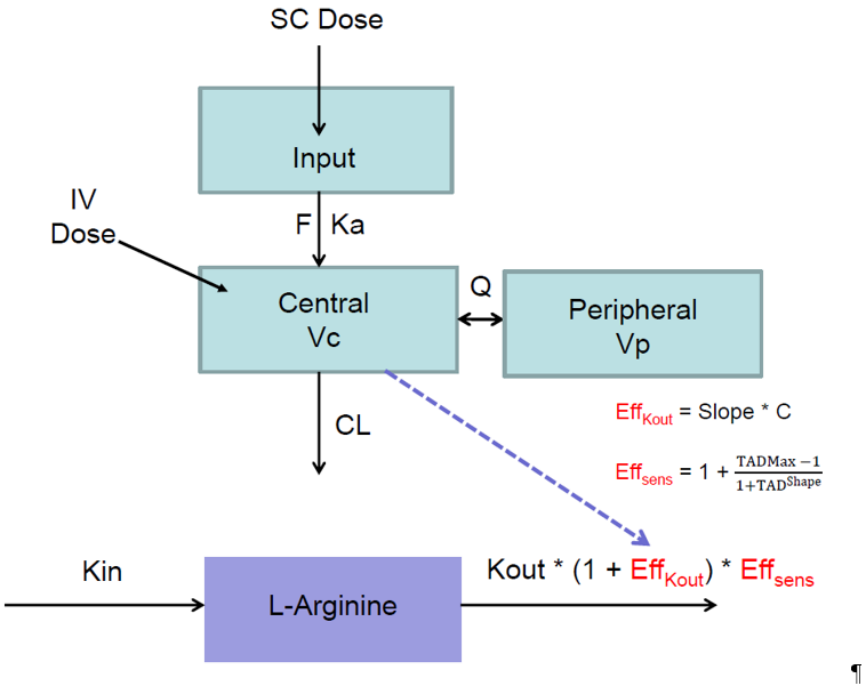
- the influence of body weight and sex is comparable following IV and SC dosing,
- following the inclusion of body weight in the PK model there is no further impact of age or sex on the PK parameters,
- the impact of anti-PEG ADAs observed during study CAEB1102-101A IV dosing represented the worst-case scenario as these are the maximum ADA effects observed in the ARG1-D pegzilarginase studies,
- the drug effect in the PKPD model is linear,
- the impact of body weight, ADA titre, and route of administration on the concentrations of L-arginine are driven wholly by their impact on the PK of pegzilarginase,
- the body weights and baseline L-arginine values in the two clinical studies are reflective of the expected population with arginase-1 deficiency,
- the 168h post-dose L-arginine concentration was available immediately and dose titration decisions could be made instantly.

The best model to fit the data was an indirect-response model with a linear drug effect and a short-term sensitivity effect (Eff_{sens}) acting on the first-order rate of plasma arginine elimination.

The PKPD model was parameterised in terms of Kout, a linear slope for drug effect, maximum Eff_{sens} on Kout (TAD Max), and the shape parameter. The sensitivity effect was considered needed to describe the rapid onset of response and is an empirical function multiplying Kout to describe better the nadir that is reached <4 hours post-dose, and the effect becomes negligible after 24 hours post-dose (Figure 4).

Baseline arginine was identified as a significant covariate on Kout. None of the other covariates evaluated (age and body weight) was significant in the PKPD model. Individual estimates of shrinkage for Kout, the drug effect slope, and TAD Max, were 11%, 3.5%, and 17.3%, respectively.

Figure 4 Schematic of the final population pharmacokinetic-pharmacodynamic model (Study 265402)



Abbreviations: CL=clearance; Eff_{Kout}=drug effect stimulation Kout; Eff_{sens}=short-term sensitivity effect on Kout; F=bioavailability; IV=intravenous; Ka=first-order absorption rate constant; Kin=zero-order rate of arginine production; Kout=first-order rate of arginine elimination; Q=intercompartmental clearance; SC=subcutaneous; Shape=shape parameter for Eff_{sens}; TAD=time after dose; TAD Max=maximum Eff_{sens} on Kout; Vc=central volume of distribution; Vp=volume of the peripheral compartment.

Note: Baseline=Kin/Kout

Source: Study report 265402, Figure 43

- **Simulations posology**

Pegzilarginase doses of 0.05 to 0.2 mg/kg QW are predicted to result in plasma arginine remaining <200 µM for almost the entire dosing interval (>98%), with similar levels of control regardless of dosing strategy. A few subjects (<3%) may require a dose of >0.2 mg/kg for arginine control using the titration rules above (168-hour post-dose plasma arginine >50 µM and <150 µM).

An IV starting dose of 0.1 mg/kg QW with dose increments of 0.05 mg/kg was selected, as this titration scheme is expected to provide optimal control of arginine for most subjects while minimising dose titrations.

Subcutaneous dosing is predicted to achieve tighter control of arginine concentrations over the dosing interval compared to IV dosing, i.e. less time <40 µM and longer in the defined normal range of 40 to 115 µM.

For both IV and SC administration, the 168-hour post-dose plasma arginine value correlates with the control of plasma arginine during the dosing interval and can be used for monitoring and dose titration decisions. A 168-hour post-dose arginine plasma concentration of 100 to 200 µM (i.e. approximately ULN to 2 times the ULN) appeared to minimize the time that arginine remained below the lower limit of the normal range (<40 µM) while maximising the time that arginine remained in the normal range (40

to 115 µM) and below the guideline recommended level (<200 µM). A dose increment of 0.05 mg/kg allows for more flexibility in dosing; however, it requires more dose titrations.

SC dosing provides greater control of arginine compared to IV dosing (less time below the lower limit of the normal range and more time in the normal range). A 168-hour post-dose arginine concentration of 100 to 200 µM (i.e. approximately ULN to 2 times the ULN) is expected to minimize the time that arginine remains below the lower limit of the normal range while maximising the time that arginine remains in the normal range and below the guideline recommended level. The impact of 1 or 2 missed pegzilarginase doses was transient, and the profiles of pegzilarginase and arginine concentrations recovered within 2 dosing intervals of restarting treatment.

The dosing strategy of pegzilarginase is aimed at maintaining the patients within the normal range for the maximum period of time over the dosing interval, i.e. minimize time outside both the lower limit of normal and the upper limit of normal. The proposed dosing strategy (QW) was associated with wide variability in the arginine levels achieved. For the proposed dosing regimen, the median simulated percentage time in range for arginine (normal arginine range - 40-115) for pegzilarginase administered via the intravenous route was 52.1% (90%PI - 27.6-63.1%) and via the subcutaneous route 78.4% (90%PI - 26.2-99.2%).

The normal range of arginine was 40-115uM in the clinical studies. The dosing algorithm used a range of 50 to 150 µM for the 168-hour post dose arginine concentration instead of the normal range of 40 to 115 µM for the following reasons:

- To account for the limits of the analytical method (WIL-317502) used to assay arginine levels within approximately 20%;
- To minimize the need for dose changes during the study;
- To maximize the time within the target range. A slightly higher threshold than the high normal value (~1.3 x the upper limit) was used given the limitations of the assay while maintaining levels within the guidelines;
- To minimize periods of hypoargininaemia. A slightly higher threshold over the value for the low normal value (~1.3 x the lower limit) was used given the limitations of the assay while preventing long periods of levels below normal. This would be the highest value over the dosing interval and using a lower value would allow longer periods of time below the normal range.

Absorption

The pharmacokinetics (PK) of pegzilarginase have been investigated in paediatric and adult subjects with ARG1-D in three studies. No healthy volunteer studies were conducted. In study 101A pegzilarginase was administered as IV formulation. In study 102A and 300A, patients received pegzilarginase as IV formulation, and pegzilarginase was also administered as SC formulation.

Pegzilarginase has been administered by slow IV injection via infusion (in approximately 30 minutes) and SC. The 30-minute slow infusion has been selected to reduce the incidence of hypersensitivity reactions. The 30-minute infusion slower infusion rate with a more dilute larger volume of 40 mL was associated with a lower incidence of hypersensitivity reactions.

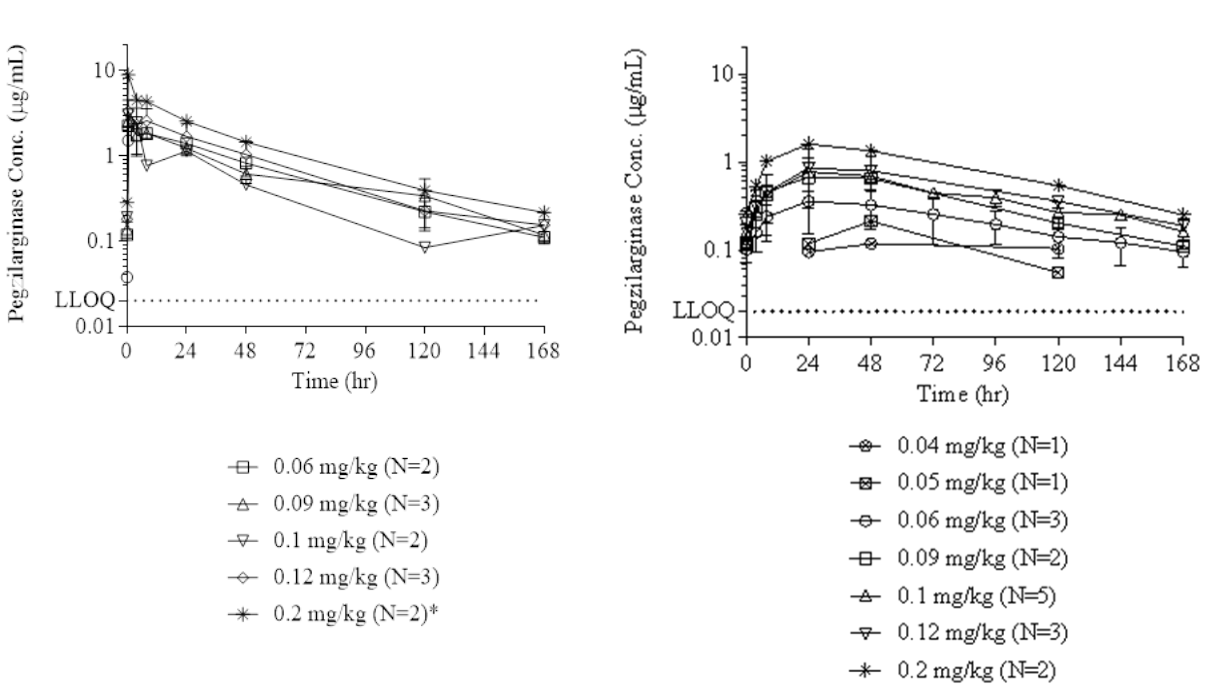
The recommended starting dose of pegzilarginase is 0.1 mg/kg, the dose, according to the SmPC, may be increased or decreased in 0.05 mg/kg increments, given every week. The drug must be diluted to the desired concentration for IV administration and infused over at least 30 minutes. Across all studies, pegzilarginase t_{max} occurred quickly after a single IV infusion, with median $t_{max} \leq 2$ hours in all cases after IV administration. Pegzilarginase t_{max} was approximately 41 hours after SC administration. Less

fluctuation between C_{max} and C_{trough} was observed following SC administration as compared to IV administration.

Pegzilarginase PK exposures (C_{max}, AUC_{0-168}) increased with increasing dose, whether the administration was by the IV or SC route. The increase in exposure was approximately in proportion to the increase in dose for both routes. Increases in mean AUC_{0-168} appeared approximately dose proportional within 0.04 to 0.2 mg/kg IV dose range and within the 0.06 to 0.2 mg/kg SC dose range.

In the long-term extension study CAEB1102-102A, the recommended dosing schedule for pegzilarginase was: IV administration QW for Weeks 1 through 24 and SC administration QW for Weeks 25 through 48. The exposure after SC administration was lower than observed after IV administration; the median absolute bioavailability (F) was 54% with ranges from 33.1% to 75.8%, and no consistent trends concerning dose level. Following IV infusion, the median t_{max} generally occurred at, or just after, the end of infusion (≤2.2 hours) and following SC administration, the median t_{max} was observed between 24 and 48 hours post-dose (Figure 5).

Figure 5 Mean (± SD) pegzilarginase concentration profiles after once-weekly administration of pegzilarginase for at least 24 weeks (Study CAEB1102-102A)

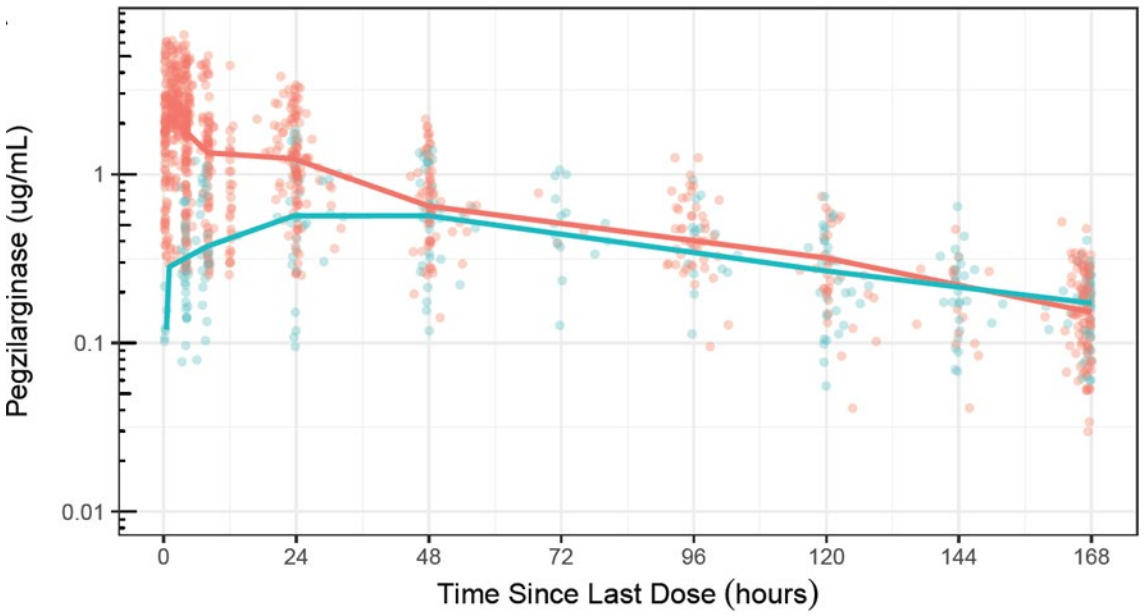


Abbreviations: C_{max}=maximum concentration; Conc.=concentration; hr=hour; LLOQ=lower limit of quantification; SD=standard deviation.
* The theoretical C_{max} at 0.2 mg/kg is 4.65 µg/mL, assuming a human serum volume of 43 mL/kg.
Note: For subjects with more than 1 subcutaneous plasma arginine profile at a particular dose level, the individual data were averaged prior to generating an average across all individuals per dose level.

The SC and IV routes of administration were also compared using the population PK model. The PopPK included a depot compartment from which pegzilarginase was absorbed into the systemic circulation. The PopPK model-estimated F following SC dosing was 58%. The 168-hour post-dose pegzilarginase concentration was similar between IV and SC dosing.

Concentration-time profiles following IV and SC dosing (IV data from all studies, SC data from CAEB1102-102A only) are displayed in **Figure 6.**

Figure 6 Concentration-time profiles by route



To allow once-weekly administration, PEG has been used as a carrier to prolong the half-life of pegzilarginase compared to endogenous arginase. The quality specifications of pegzilarginase allow PEG:protein ratios should be between 6 and 12. During clinical development, pegzilarginase batches with a different degree of pegylation have been evaluated. The data are limited as most subjects received batches with a PEG: protein ratio of 8. A few subjects with available IV PK data, received pegzilarginase from batches with different PEG: protein ratios. No clear difference in the Cmax and AUC was observed between subjects receiving batches with PEG: protein ratios of 11 and 8. The limited data suggest no PK differences between batches with a different degree of pegylation.

In the clinical studies, subcutaneous (SC) injection sites used included left and right arm, left and right thigh, abdomen, left and right, upper and lower quadrant. Limited PK data are available to evaluate the potential effect of different sites of SC injection within individuals. Thigh:arm and abdomen:arm ratios for 2 evaluable patients suggest that the PK is comparable between injection sites. The company did not present a between-subject comparison of different injection sites. In literature a high risk of injection site-dependent SC absorption is mainly observed for compounds with a high molecular weight (≥ 16 kDa) and fast absorption (t_{max} < 2 hours) or fast elimination (CL/F ≥ 39 L/h). Based on literature, the different sites of SC injection are expected to have a low impact on the absorption, as pegzilarginase is a large molecule which is slowly absorbed from the subcutaneous compartment (t_{max} of 30-50 hours) and has a low clearance (CL/F) of 1.6 mL/h/kg (0.112 L/h for 70kg adult).

The impact of a missed dose has been evaluated using population PK model simulations. The impact of one or two consecutive missed doses on the profile of pegzilarginase and L-arginine was transient, with the profile returning to the expected value.

Food effect is not relevant, as pegzilarginase is administered by intravenous infusion or subcutaneous injection.

Distribution

Pegzilarginase is a modified enzyme therefore is not expected to bind to plasma proteins. Thus, the risk of protein displacement and interaction with drugs highly bound to the plasma proteins is neglected.

Across all studies, pegzilarginase mean volume of distribution (Vss) was low. Following IV administration of 0.05 and 0.2 mg/kg the Vss was approximately 47 mL/kg (range: 33 to 59 mL/kg; 1.0 – 1.8l in a 31 kg patient), which is similar to human serum volume. Following SC administration Vss was approximately 164 mL/kg (range: 111 to 237 mL/kg, 3.4 – 7.3 in a 70 kg patient), about 3.5-fold higher.

It appears that the differences in volume of distribution are the result of problems with adequate characterisation of terminal phase of elimination after SC injection. The popPK analysis included 2 compartments, with a Vc of 1.33 L and a Vp of 0.438 L and the volume of distribution was the same for both routes of administration. The obtained data suggest that pegzilarginase is mainly distributed in the vascular system.

Based on average molecular weight and charge distribution profile, pegzilarginase is not expected to cross the blood-brain barrier.

Elimination

Across all studies, pegzilarginase mean CL after IV administration at 0.05 to 0.2 mg/kg was approximately 1 mL/h/kg (range: 0.6 to 1.9 mL/h/kg) and was unaffected by dose level or repeat administration. The mean $t_{1/2}$ after IV administration was approximately 37 hours (range: 16 to 56 hours) from NCA analysis.

The pegzilarginase mean apparent clearance (CL/F) after SC administration at 0.06 to 0.2 mg/kg was approximately 1.6 mL/h/kg (range: 1.3 to 1.9 mL/h/kg). The mean $t_{1/2}$ after SC administration appeared longer (approximately 62 hours; range: 51 to 78 hours) than after IV administration from NCA analysis.

The applicant presented a comparison of the terminal elimination half-lives for IV and SC administration based on the NCA data and simulated the IV and SC concentration-time curves for a typical subject using the popPK model. The NCA data appear to indicate that the t1/2 for the SC formulation is longer than that for the IV formulation, indicative of some flip-flop kinetics behaviour. The observed half-life was about 40 hours following multiple IV doses and about 60 hours following multiple SC doses. Based on pop PK modelling, pegzilarginase has a half-life of about 50 hours; when the estimated CV of 25.2% is taken into account, the observed half-lives of 40 and 60 hours are within the range of population PK simulations.

The PopPK model-estimated CL was 0.029 L/h, with a total (Vc+Vp) pegzilarginase volume from the PopPK model of 1.77 L. This indicates a model-estimated elimination $t_{1/2}$ of approximately 50 hours, independent of the route of administration.

Pegzilarginase is a recombinant human enzyme and is expected to be metabolically degraded through proteolysis into small peptides and amino acids. No mass balance study has been conducted.

Dose proportionality and time dependencies

Pegzilarginase has been studied in a dose range of 0.015-0.20 mg/kg IV and 0.06-0.2mg /kg SC. The increases were approximately dose-proportional over a dose range of 0.04 to 0.2 mg/kg IV and 0.06 to

0.2 mg/kg SC. Limited accumulation has been observed following IV administration of pegzilarginase. Time dependency has only been studied following IV administration and not following SC administration. The mean $t_{1/2}$ was approximately 37 h, range 16 to 56 hours following IV administration and 62 hours; range: 51 to 78 hours following SC administration. The C_{max} and AUC_{0-168} values were similar after repeated doses of pegzilarginase (after 1, 12 and 24 weeks). Overall, single dose and at steady state data indicate that pegzilarginase PK is time-independent.

Pharmacokinetics in the target population

The PK in the target population is summarised in Table 3. Presented PK parameters were derived using the final population PK model; simulated dose in the model was 0.1 mg/kg for 5 weeks for a patient with a body weight of 31 kg.

Table 3 Pharmacokinetic parameters at steady state based on the population PK model

	Pegzilarginase	
	Intravenous	**Subcutaneous**
General information		
Steady state exposure [C_{max} (µg/ml)]*	2.48 (19.9%)	0.579 (19.9%)
Steady state exposure [AUC_{0-168} (h*µg/ml)]*	108 (18.3%)	61.3 (18.3%)
Dose proportionality	Pegzilarginase exposures increase in an approximately dose-proportional manner with linear PK	
Accumulation	Negligible accumulation after weekly dosing	
Absorption		
T_{max} (h)**	0.25^	34 (22.0 - 46.0)
Bioavailability*	-	56.9% (21.2%)
Distribution		
Vc (ml)+	1350 (41.6%)	
Vp (ml)+	447 (38.3%)	
Elimination		
t½ (h)	50.2++(7.53%)	
CLss (ml/h)	29.6 (42.8%)	29.6 (42.8%)
Metabolism		
Pegzilarginase is a recombinant human enzyme and is expected to be metabolised into small peptides and amino acids by catabolic pathways		

Abbreviations: AUC_{0-168}=area under the concentration-time curve from time 0 to 168 hours; CLss=clearance ;C_{max}=maximum observed concentration; IV=intravenous; SC=subcutaneous; t½=half-life; T_{max}=time to maximum concentration; Vc= volume of the central compartment; Vp=volume of the peripheral compartment

* Data displayed are geometric mean and geometric CV(%)

** Data displayed as [median (range)]

+ The model comprised two-compartments (central and peripheral) with 2 separate volumes

++ t½ displayed is the calculated t½β.

^ For IV dosing, the T_{max} corresponds to the time of the first measured PK sample. In these simulations the first PK sample was set at the end of infusion (0.25 h post-dose) for all subjects with no variability.

Special populations

The PK of pegzilarginase has only been studied in the target population: patients with arginase-1 deficiency. In the population PK model, body weight and anti-PEG ADA were significant covariates.

Overall, body weight had a minimal impact (<20%) on the exposure of pegzilarginase. As the pegzilarginase dosing is weight based, it had no clinically meaningful effect on PK.

The applicant evaluated the effect of anti-PEG ADA and anti-pegzilarginase ADA. Anti-PEG ADA was identified as a significant covariate, anti-PEG ADA had a titer-dependent impact (>1:100) on

pegzilarginase exposure compared to the absence of anti-PEG ADA. This effect appeared to be transient, with CL of pegzilarginase recovering to the expected level within the next dosing interval. Anti-pegzilarginase ADA did not have a significant effect.

After the inclusion of weight effects in the model, no apparent trends remained for any potential effects of age. Further, sex, race, anti-pegzilarginase ADAs, CrCL, ALT, AST, ALB, and bilirubin were investigated as covariates and not found to be statistically significant after the inclusion of body weight.

Pegzilarginase utilizes a 5 kDa PEG, which is eliminated via renal glomerular filtration in patients with normal renal function.

No studies were conducted in patients with hepatic impairment; this is acceptable as pegzilarginase is expected to be degraded into inactive protein and amino acids via the standard protein elimination pathways. These pathways are usually not impaired in patients with hepatic dysfunction.

Pegzilarginase has been studied in adult subjects, adolescents of 12-17 years old and children in the age range of 2-11 years old. The oldest patient participating in the three clinical trials was 32 years old, therefore no elderly patients were included in the clinical trials. In all age groups, the total dose is based on the patient's body weight (kg). No data are available in children below 2 years of age.

The applicant presented untransformed and dose-normalised concentration-time profiles for adult subjects (n = 6) and paediatric subjects (n = 19 for 2-11y, n = 12 for 12-17y) following IV and SC dosing. Following dose normalisation, the paediatric population showed a consistently higher exposure compared to adults.

Pharmacokinetic interaction studies

Pegzilarginase is a recombinant human enzyme. Thus, the drug-drug interactions with cytochrome P450 enzymes or transporters are not expected. No interaction studies have been performed. This is acceptable; no drug-drug interactions are expected.

Pharmacokinetics using human biomaterials

No studies in human biomaterials have been performed to investigate the metabolism or potential for interactions. This is acceptable as no drug-drug interactions are expected.

2.6.2.2. Pharmacodynamics

Mechanism of action

Pegzilarginase (AEB1102, Co-Arg1-PEG) is a cobalt substituted, a PEGylated modified recombinant human arginase 1 enzyme, is an enzyme replacement therapy with an increased potency and extended half-life (t1/2) compared to the native enzyme because of its pegylation. It is intended to substitute for the deficient human arginase 1 enzyme activity in patients with ARG1D.

Primary and secondary pharmacology

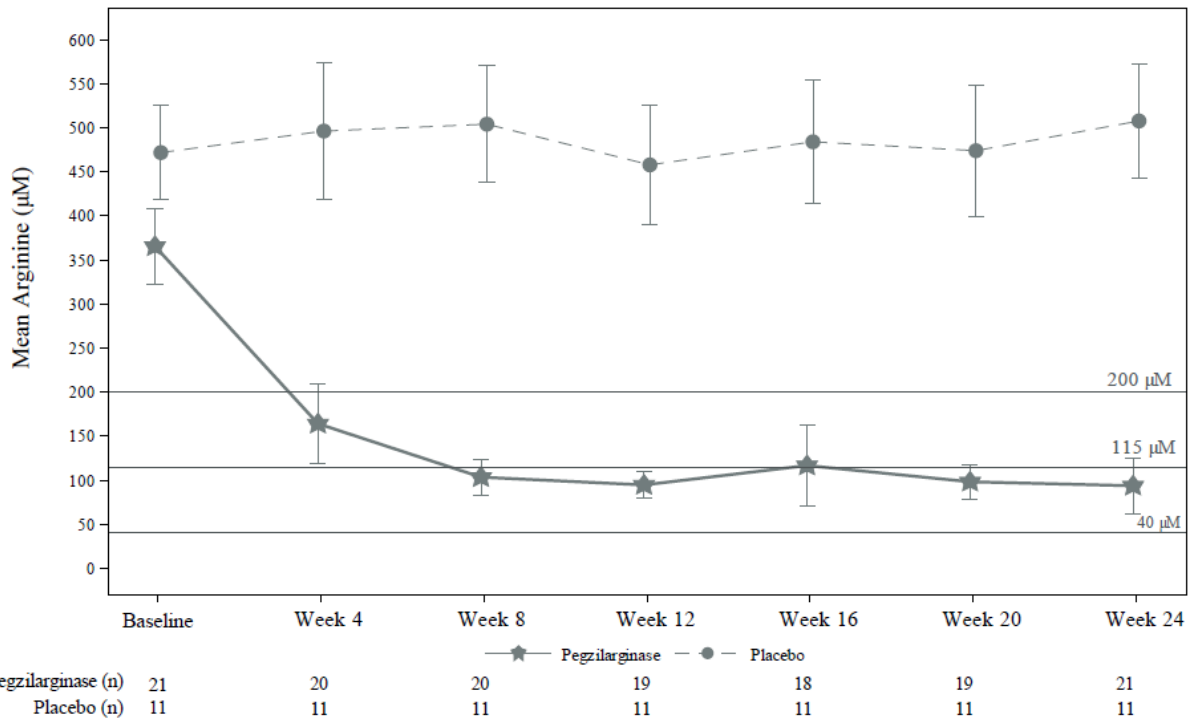
Pegzilarginase PK exposure increased in an approximately dose-proportional manner after QW IV administration. Administration of pegzilarginase demonstrated early, consistent, and sustained reductions in plasma arginine below the guideline recommended level (<200 µM), and also below the upper normal level of 115 µM for virtual all subjects. These reductions in plasma arginine were maintained after SC administration. Administration of pegzilarginase reduced plasma concentrations of GCs and increased ornithine concentrations throughout the double-blind period.

Demonstration of the lowering of arginine levels, the primary pharmacological target, was the primary objective of the main study (300A) in ARG1-D patients. Compared to placebo (n=11), pegzilarginase (n=21) significantly reduced the arginine plasma levels. The majority of the patients who received pegzilarginase achieved a reduction of arginine, as shown in Figure 7. After 24 weeks of treatment, 90.5% of subjects in the pegzilarginase group (19/21) achieved arginine values within the normal range (≥40 µM to ≤115 µM) versus none in the placebo group.

In addition, the ornithine concentration and the guanidino compound (GC) concentrations were analysed as secondary PD outcomes. Ornithine is a product of arginine enzymatic degradation, the GCs are formed via other pathways. Baseline plasma ornithine levels were below the normal range and a dose-dependent increase occurred with pegzilarginase administration. After 24 weeks of treatment, the measured plasma GCs (including α-keto-δ-guanidinovaleric acid [GVA], argininic acid [ArgA], N-α-acetyl-arginine [NAArg], and guanidinoacetic acid [GAA]) were reduced by 50-70% compared to baseline. The observed decreases in plasma GC levels corresponded to reductions in plasma arginine, supporting pegzilarginase-mediated reductions in the generation of these arginine-derived metabolites.

Given the high variability of genotypes of ARG1-D, no meaningful analyses could be performed on the relationship between genotype and PD effects.

Figure 7 Summary of least square mean (95% CI) 168-hour post dose arginine levels (µM) over time in Study 300A double-blind period



The applicant did not consider that there are off-target effects of pegzilarginase in the CTD. The trials did not signal QTc prolongation or other cardiac arrhythmias. Neither are PD interactions with other drugs expected.

Immunogenicity

An integrated pharmacokinetic (PK)-pharmacodynamic (PD)-ADA analysis was used to evaluate the impact of ADA on efficacy as measured by the magnitude of reduction in plasma arginine levels. ADAs occurred early but were transitory and were not associated with prolonged clinically relevant changes in the PK or PD of pegzilarginase.

Study samples were classified as follows:

- 'ADA positive' if samples were confirmed positive (i.e., sufficient signal inhibition: equal or above the confirmatory cut point) in either the anti-pegzilarginase or the anti-PEG assay or both.
- 'Pre-existing ADA' if ADA was present in baseline samples (i.e., before first administration with study drug in Study 101A Part 1 or Study 300A).
- 'Treatment-induced ADA' if ADA developed after initiating treatment in subjects who were ADA negative at Baseline.
- 'Treatment-boosted ADA' if there was an increase of at least 4-fold in ADA titer relative to Baseline.

The pooled incidence of ADA overall (N=48) was 25.0%, with a pooled prevalence of ADA overall of 37.5%. ADAs against PEG were reported more frequently than ADAs against pegzilarginase.

Relationship between plasma concentration and effect

The relationship between pegzilarginase concentrations and the effect on arginine levels has been evaluated using population PK-PD modelling. Baseline arginine has been identified as a relevant covariate.

The model was used to compare IV and SC dosing. A graphical representation comparing the model-predicted profile of plasma arginine concentration following IV and SC administration is presented in Figure 7. Different starting doses and dosing increments were simulated (using 2000 virtual subjects) to support dose selection for both IV and SC administration.

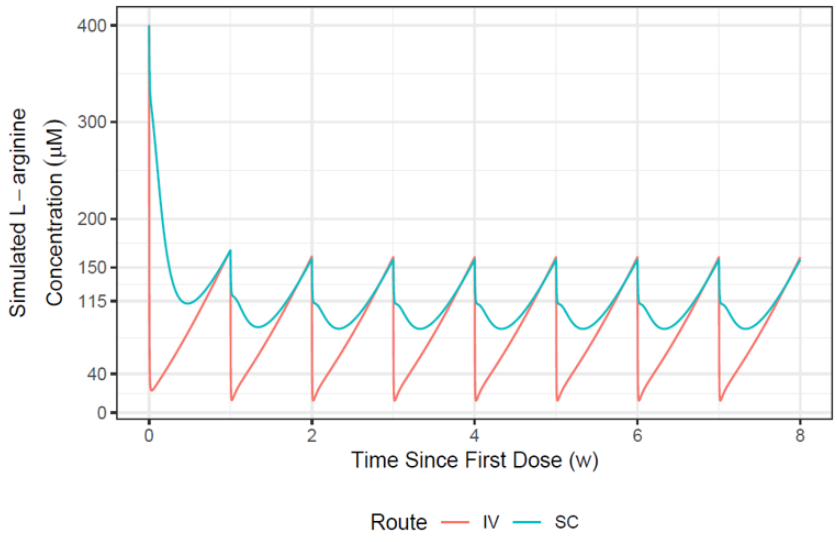
Pegzilarginase doses of 0.05 to 0.2 mg/kg QW are predicted to result in plasma arginine remaining <200 µM for almost the entire dosing interval (>98%), with similar levels of control regardless of dosing strategy. A few subjects (<3%) may require a dose of >0.2 mg/kg for arginine control using the titration rules above (168-hour postdose plasma arginine >50 µM and <150 µM).

Subcutaneous dosing, across all dosing scenarios, was predicted to achieve tighter control of arginine concentrations over the dosing interval compared to IV dosing (less time <40 µM and longer in the defined normal range of 40 to 115 µM).

Subjects with a higher baseline arginine tended to require a higher predicted final maintenance dose, regardless of the dosing strategy and regardless of dose route.

The impact of 1 or 2 missed pegzilarginase doses was transient, and the profiles of pegzilarginase and arginine concentrations recovered within 2 dosing intervals of restarting treatment.

Figure 8 Model-predicted effect of dosing route on plasma arginine concentration



¶

Abbreviations: ADA=anti-drug antibody; IV=intravenous; QW=once weekly; SC=subcutaneous; w=week.¶
Notes: Solid lines represent a simulated median concentration-time profile for 2 subjects with body weights of 31kg in the absence of any ADAs. Eight doses of 0.05 mg/kg were administered QW. One subject received IV dosing the other SC dosing.¶
Source: Study report 265402, Figure 47¶

2.6.3. Discussion on clinical pharmacology

Enzyme activity assays have been used in the clinical studies to determine the concentration of pegzilarginase in human serum samples. Different assay ranges have been used, and different laboratories have been involved. The assays were appropriately validated and cross-validated, and the change in assay range is appropriate. Within-study performance has been validated in all clinical studies.

Pegzilarginase can be administered via IV administration and via SC administration. The applicant sufficiently characterised and compared the pharmacokinetics of pegzilarginase via both dosing routes. Simulations suggest that subjects may transition to subcutaneous administration using the same intravenous dose. The model predicts similar trough concentrations for IV and SC dosing.

The PK of pegzilarginase has been studied in the target population using population PK modelling and the special populations were evaluated using the population PK. Pegzilarginase has not been studied in patients with renal impairment. As pegzilarginase utilizes a 5 kDa PEG, it cannot be excluded that in patients with impaired renal filtration the clearance of PEG moieties even of smaller size by glomerular filtration may be hampered which may lead to PEG internalisation within the cells.

Pharmacodynamics of pegzilarginase

Pegzilarginase cleaves excess plasma arginine to ornithine and urea, the natural breakdown products of arginine, and represents the first potential enzyme therapy for patients with arginase 1 deficiency (ARG1-D). The result of the administration of the pegzilarginase to patients with hyperargininaemia is lowering the plasma excess of arginine to normal levels (at least below 200uM). At the same time, the restoration of the ornithine level is expected. Furthermore, reduction of arginine also reduces the production of the toxic guanidino compounds.

Treatment with pegzilarginase significantly lowered plasma arginine to the recommended guideline of <200 µM in all subjects, with 93% (13/14) of subjects maintaining this at the end of 8 weeks of IV QW

administration and 43% (6/14) achieved levels <115 µM at this time point. Plasma ornithine increased, and plasma GCs were reduced in accordance with decreasing plasma arginine levels. Pegzilarginase PK exposure appeared to increase approximately dose-proportionally after IV or SC administration, whether a single dose or after repeating QW dosing.

Administration of pegzilarginase demonstrated consistent and sustained reduction in plasma arginine and GCs. Administration of pegzilarginase showed consistent and sustained increases in plasma ornithine. These reductions in plasma arginine and GC levels and increased plasma ornithine levels were consistent and maintained after SC administration. ADAs were observed in 37.5% of subjects with ARG1-D (with 25.0% experiencing treatment-induced ADA). ADAs that occurred early after treatment initiation were transitory and resolved during continued treatment. No QT study has been conducted, which is acceptable, given the mode of action and as there was no signal from the clinical trials of ECG abnormalities.

2.6.4. Conclusions on clinical pharmacology

The pharmacokinetics (PK) of pegzilarginase have been investigated in 37 paediatric and adult subjects with ARG1 deficiency. Despite the limited number of subjects, the pharmacokinetics of pegzilarginase has been appropriately investigated using non-compartmental analysis and population PK methods.

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

Pegzilarginase dose is titrated based on arginine levels, and monitoring is required. During the clinical trials, specifically prepared tubes with an arginase enzyme blocker were used to inhibit residual pegzilarginase activity and stabilize arginine in the plasma samples. The applicant committed to make the tubes with the enzyme blocker nor-NOHA commercially available, which is supported.

After 24 weeks of treatment with pegzilarginase, the GC levels were significantly reduced by 50-70% from baseline and as compared to placebo. However, for three of the four measured compounds (α-K-δ-GVA, α-NAA, Arg-A) the plasma levels were still above the upper level reported for the normal reference population. Thus, the clinical relevance on the PD effect regarding the reduction of GC levels remains unclear. It was accepted that the final study report on the GCs will be provided in the post-marketing setting.

2.6.5. Clinical efficacy

The clinical development programme consisted of three clinical studies, in which 48 patients with arginase 1 deficiency (ARG1-D) and hyperargininaemia received pegzilarginase treatment. In all studies, patients continued their individual prior treatment with a protein-restricted diet and nitrogen scavengers if applicable.

Study CAEB1102-300A (Study 300A) was a 24-week randomised, double-blinded, placebo-controlled study in 32 paediatric and adult patients receiving weekly IV doses (21 pegzilarginase, 11 placebo). The randomised part of the study is completed. All patients who finished the 24 weeks randomised part were eligible for the long-term extension (LTE) study with pegzilarginase, which is still ongoing. The first 8-weeks of the LTE remained blinded. After 8 weeks in the LTE, patients could be switched to SC dosing in the open-label extension study.

Study CAEB1102-101A (Study 101A) is a Phase 1 /2 open-label uncontrolled dose-finding study in 16 paediatric and adult patients with ARG1-D. In part A, single IV doses were individually titrated every two weeks to an arginine target level of < 200 uM, followed by part B, where treatment was continued a weekly IV dosing for 7 weeks.

From this study, 14 subjects entered the open-label extension study 102A, which is still on-going. In the extension study, patients could be switched to SC weekly dose after 24 weeks if IV dosing.

For an overview of the studies, see Table 4 below.

Table 4 Summary of current pegzilarginase clinical study programme

Protocol No. Study Start – Stop Date	Total Enrolled Age Range	Study Objective	Study Design	Main Criteria for Inclusion	Weekly Dose	Duration of Treatment	Primary Objective
CAEB1102-300A (multicentre; ongoing) 01 May 2019 – ongoing[a] US: 9 sites, CND: 1, UK: 4, FR: 2, DE: 1, AU 1, IT 1 site	N=32 M: 19 F: 13 Age: ≥2 yrs	Efficacy and safety	R (2:1), DB, PC	Subjects aged ≥2 years with a diagnosis of ARG1-D	0.05, 0.10, 0.15, and 0.20 mg/kg	24 weeks double-blind (IV) and up to approximately 150 weeks (IV+SC)	Change from Baseline in plasma arginine after 24 weeks of study treatment
CAEB1102-101A 25 Aug 2016 – 28 Feb 2019 (US: 6 CND: 1 , PT 1 , UK: 1 site)	N=16 M: 5 F: 11 Age: 5-31 yrs	Safety and tolerability and efficacy[c]	NR, OL, single ascending dose (Part 1), repeated dosing (Part 2)	Subjects aged ≥2 years with a diagnosis of ARG1-D	Part 1 doses: 0.015 to 0.20 mg/kg (2-week intervals) Part 2: doses: 0.015 to 0.20 mg/kg	Part 1: up to 7 doses (IV) over a maximum of 14 weeks Part 2: up to 8 doses (IV) over 7 weeks	Safety and tolerability
CAEB1102-102A 07 Dec 2017 – ongoing[b] US: 6 CND: 1 , PT 1 , UK: 1 site	N=14 M: 3 F: 11 Age: 6-32 yrs	Long-term safety, tolerability, and efficacy	OL	Completion of Study 101A with tolerance to dosing of pegzilarginase and meet applicable eligibility criteria from the original study.	0.03 to 0.20 mg/kg	Up to 4 years (IV+SC)	Safety and tolerability

Abbreviations: ARG1-D=arginase 1 deficiency; AU = Austria; CND = Canada; CSR=clinical study report; DB=double-blind; DE = Germany; F=female; FR = France; IT =Italy; IV=intravenous; LTE=long-term extension; M=male; NR=non-randomised; OL=open-label; PC=placebo-controlled; PT = Portugal; R=randomised; SC=subcutaneous.

a Data cutoff date for Study 300A: 14 October 2021.

b Data cutoff date for Study 102A: 11 June 2021.

c Efficacy was evaluated in the secondary and exploratory objectives.

2.6.5.1. Dose response studies

Study **CAEB1102-101A** was an exploratory dose-finding study.

The primary objective of this study was to evaluate the safety and tolerability of intravenous (IV) administration of AEB1102 (pegzilarginase, primarily referred to as study drug hereafter) in subjects with hyperargininaemia/ARG1-D. The secondary objective was measuring PK and PD and the effect of the drug on arginine levels. Exploratory objectives were measuring effects on diverse neurocognitive,

developmental, and quality of life (QOL) and motor-function scales and the frequency of disease-related symptoms such as seizures, hyperammonaemic episodes and transaminase elevations.

This open-label study was conducted in two parts: Part 1 (Single Ascending Dose Escalation, 14 weeks, maximal 7 doses every-other-week till the target arginine level was achieved) and Part 2 (Repeated Dosing 7x a QW dose).

Patients above 2 years of age were eligible with a confirmed diagnosis of ARG1-D (by genotyping or deficient RBC arginase), with plasma arginine levels from one or more of 3 baseline samples >250 µM or 2 of 3 baseline samples >200 µM, and who had no episode of hyperammonaemia in the period of 6 weeks before inclusion. A sample size of at least 10 paediatric and adult subjects was determined using clinical rather than statistical considerations.

Each subject received escalating doses of the study drug in Part 1 with a 2-week washout/observation period between each successive dose level. The possible doses for each subject in Part 1 were 0.015, 0.03, 0.06, 0.10, 0.15, 0.20, and 0.30 mg/kg, as needed to optimize plasma arginine. At first, patients above the age of 12 were included, followed by the younger age groups.

A dose level could be repeated based on the investigator decision till the end of the study period (14 weeks).

Part 2 was a repeat-dosing period for subjects who completed Part 1 without a clinically significant reason to preclude continued dosing of the study drug. The starting dose level for Part 2 was based on the PK of the study drug and plasma arginine levels from single- and repeat-dose results available at the time the subject completed Part 1.

Subjects were instructed to continue their prescribed diet and medications without modification for the duration of the study. During each Baseline in Parts 1 and 2, prior to each dose of study drug during Part 1, and prior to doses on Weeks 4 and 8 during Part 2, subjects completed a 3-day diet record using a diary to collect the information.

Adverse events and discontinuations were the primary variables. PK, PD (arginine, Guanidino Compound Levels) were secondary outcomes. The plasma samples were also analyzed for anti-pegzilarginase and anti-PEG antibodies throughout the study. According to the protocol, extra samples were also drawn within three hours after a hypersensitivity event to monitor ADA's and Complement 3. A large range of clinical endpoints were explored on neurocognitive, motor, and QOL domains. Some of the above assessments were added or deleted in protocol amendments.

PD Outcomes Study 101A Part 1; single dose titration

For single doses of 0.015, 0.03, 0.06, 0.1, and 0.2 mg/kg, the mean percent change from Baseline of plasma arginine was -21.1% (n=16), -26.7% (n=15), -41.6% (n=10), -49.2% (n=4) and -77.4% (n=1), respectively, indicating a PK-PD dose-response relationship. No durable satisfactory PD response was shown for the lowest 0.015 mg/kg dose. For the intermediate level dose steps of 0.03, 0.06, 0.1 mg/kg, the arginine levels were reduced below the clinical target level of < 200 uM Arginine in approximately 25% of the subjects per dose step.

Only one subject was up-titrated to the maximum allowed dose of 0.2 mg/kg, and only in this subject, the arginine levels were below the most critical target level of 115uM (ie. the upper level of normality in healthy humans), for a prolonged period of 168 hrs after IV infusion.

Outcomes Study 101A Part 2, repeat dosing

About 50 percent of the subjects underwent dose adjustments to achieve further improvements in plasma arginine control in this part of the study. At the end of this 8 weeks study, the distribution of the doses was as follows: 6 subjects received doses around 0.05 mg/kg (0.04-0.06 mg/kg), 7 subjects received doses around 0.1 mg/kg (0.09-0.12) and one a dose of 0.2 mg/kg. The majority of the subjects (80%) achieved the target level of < 200uM at the last 4 doses, and about 50% also the more critical target of <115 uM.

All subjects had increased levels of guanidino compounds as GVA, ARGA, and NAA at baseline, which are the toxic metabolites of Arginine. However, these levels continue to drop from baseline.

ADA formation Study 101A, Part 1 and 2

Two out of 16 subjects (12.5%) were ADA-positive at baseline prior to study drug administration, and 8 of 16 (50%) became ADA-positive (both anti-PEG and anti-pegzilarginase) after pegzilarginase exposure in Part 1 of the study. Overall, there was a trend for reduced PD effect (i.e. arginine reduction) in the ADA-positive subjects, particularly at the low dose levels.

The rate of ADA positivity declined at repeat dosing. No subjects were ADA-positive at the end of Part 2 of this study.

Safety Study 101A, Part 1 and 2

In Part 1, the dosing was temporarily interrupted for 6 patients (5 after 0.03 dose and one after the 0.06 dose), and this was due to hypersensitivity in two cases (both ADA-positive). Hypersensitivity (n=3) and hyperammonaemia (n=6) were reported as SAEs. The subjects with hypersensitivity were able to complete the infusion using concomitant medications (antihistamines) and reducing the infusion rate. Pre-medications like antihistamines and steroids were used for some subsequent administration of the study drug, and all subjects could continue using pegzilarginase.

All patients with a hyperammonaemia event in this study had a previous history of hyperammonaemia before entering the study. For 2 cases, the investigator discontinued the concurrent treatment of ammonia scavengers they had already received before the study, which did not conform to the protocol.

Efficacy Study 101A, part 2

Clinically meaningful improvements (meaningful was predefined) in at least 1 clinical outcome measure in 9 of 14 subjects who completed Part 2.

The most notable effects were on motor function tests like 6MWT (4 MCID responders), GMFM Part E, which assesses walking, running, and jumping (2 responders), and PROMIS Physical Functioning (3 responders). Since the response was very heterogenous over the multiple outcomes and given the open-label design of the study, no definitive conclusion of efficacy could be drawn.

Conclusions dose finding study 101A

Overall, the study supported the proof of concept of pegzilarginase to reduce arginine levels below the treatment target.

Based on the study's outcomes, an initial dose of 0.1 mg/kg IV was chosen for the main study 300A, which could be individually titrated based on arginine levels, with dosing steps of 0.05 mg/kg to a dose range of 0.05-0.2 mg//kg.

A later development in the clinical programme was the SC dose. As SC dosing was first applied in the long-term extension study 102A, this will be discussed in the section 'supportive studies' below.

2.6.5.2. Main study

PEACE (PEGZILARGINASE EFFECT ON ARGINASE 1 DEFICIENCY CLINICAL ENDPOINTS), study code CAEB1102-300A

Methods

- **Study design**

This study (Study 300A) was a multi-centre, randomised, DB, placebo-controlled study designed to evaluate the safety and efficacy of pegzilarginase and planned to be conducted in approximately 30 subjects with ARG1-D, with the total duration of the study expected to be approximately 178 weeks per subject. This study consisted of the following periods (Figure 9):

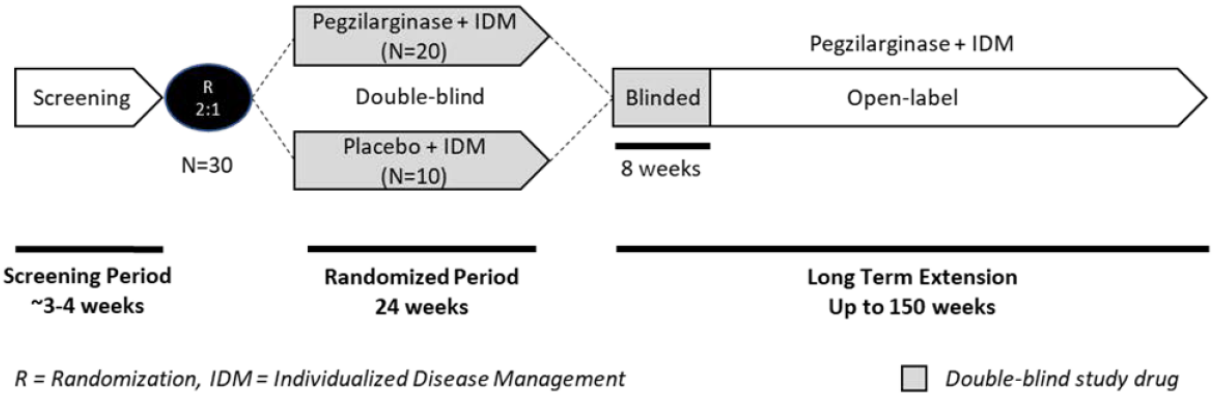
1. A screening period of 3 to 4 weeks in duration to collect all necessary information to ensure the subjects met study eligibility criteria and to establish Baseline plasma arginine data, collect prescribed diet data, and determine adherence to the prescribed diet using a diet diary.

2. A randomised DB period of 24 weeks.

3. An open-label LTE period of up to approximately 150 weeks in which all subjects received active pegzilarginase. The first 8 weeks of treatment previously administered during the DB period were to remain blinded to ensure that study data relating to the randomised period is collected prior to unblinding.

Subjects were randomly assigned to treatment following completion of all screening assessments and confirmation of study eligibility in a 2:1 ratio to receive QW IV infusions of pegzilarginase on top of IDM (individualised disease management) or placebo plus IDM during the 24-week DB period. Randomisation was stratified by the severity of prior history of hyperammonaemia.

Figure 9 Schedule of Study 300A[#]



[#]Note: in reality, 21 subject were assigned pegzilarginase, and 11 to placebo.

IDM (individualised Disease management) prior to the study was maintained unchanged during the trial. Subjects were required to maintain dietary protein intake levels that were consistent with their Baseline levels for the entire duration of the randomised DB AND the 8-week blinded period of the LTE period of the study.

The study is still on-going. Only the randomised 24-week study phase is completely finished. The final results will be provided post-marketing, which is acceptable.

- **Study Participants**

Inclusion criteria:

1. Informed consent of caretakers or patient.

2. A current diagnosis of ARG1-D as documented in medical records, which included 1 of the following: elevated plasma arginine levels, a mutation analysis that resulted in a pathogenic variant, or RBC arginase activity. For entry into this study, subjects also had to fulfil the following plasma arginine criteria:

a. The average of all measured values of plasma arginine during the Screening Period prior to the randomisation visit (Visit 1, Study Day 1) was ⩾250 μM.

b. If a subject was re-screened, the only values that were considered for eligibility assessment were those in the current Screening Period.

3. Subjects were ⩾2 years of age on the date of informed consent/assent.

4. The subject was assessable for clinically meaningful within-subject change (clinical response) on at least 1 component of 1 assessment included in the key secondary/other secondary endpoints. To be considered assessable, the subject was able to complete the assessment and had a Baseline deficit in at least 1 component as defined in Table 5.

Table 5 Definition of baseline deficits for key secondary endpoint

Domain	Assessment	Component	Definition of Baseline Deficit		
Mobility	Timed Walk Test	2MWD* (meters)	Definition of Baseline deficit for 2MWT varies by age and sex.		
			Age	Female	Male
			3-5	<112.9	<110.6
			6-8	<155.8	<154.9
			9-11	<172.0	<169.9
			12-15	<168.7	<172.1
			16-17	<167.5	<173.4
			≥18	<142.4	<148.8
	GMFM†	Part D	<35		
		Part E	<68		

2MWT = 2-Minute Walk Test; 2MWD = 2-Minute Walk Distance; GMFM = Gross Motor Function Measure
* Definition of Baseline deficit is calculated from the NIH Toolbox motor domain dataset (2-minute Walk Endurance Test).
† Definition of Baseline deficit is from Oeffinger 2008.

5. Have received documented confirmation from the Investigator and/or dietician that the subject could maintain their diet in accordance with dietary information presented in the protocol (i.e., could maintain the current level of protein consumption, including natural protein and EAA supplementation).

6. Subjects receiving ammonia scavenger therapy, anti-epileptic drugs, and/or medications for spasticity (e.g., baclofen) were on a stable dose of the medication for at least 4 weeks prior to randomisation and were willing to remain on a stable dose during the DB portion and blinded follow-up portions of the study.

7. Female subjects of childbearing potential had a negative serum pregnancy test during the Screening Period before receiving the first dose of study treatment, and a negative urine pregnancy test on the day of the first dose, prior to the first dose. If the subject (male or female) was engaging in sexual activity that could lead to pregnancy, he or she were surgically sterile, postmenopausal (no menses for 12 months without an alternative medical cause or a high follicle-stimulating hormone level in the postmenopausal range in women not using hormonal contraception or hormonal therapy), or agreed to use a highly effective method of birth control during the study and for a minimum of 30 days after the last study treatment administration.

Exclusion criteria:

1. Had a hyperammonaemic episode (defined as an event in which a subject had an ammonia level $\geqslant$ 100 μM with 1 or more symptoms related to hyperammonaemia requiring hospitalisation or emergency room management) within the 6 weeks before the first dose of study treatment was administered.

2. Had an active infection requiring anti-infective therapy within 3 weeks prior to first dose.

3. Had known active infection with human immunodeficiency virus, hepatitis B, or hepatitis C.

4. Had extreme mobility deficit, defined as either the inability to be assessed on the Gillette Functional Assessment Questionnaire (GFAQ) or a score of 1 on the GFAQ.

5. Had other medical conditions or comorbidities that, in the opinion of the Investigator would interfere with study compliance or data interpretation (e.g., severe intellectual disability precluding required study assessments).

6. Had participated in a previous interventional study with pegzilarginase.

7. Had a history of hypersensitivity to polyethylene glycol (PEG) that, in the judgment of the Investigator, put the subject at unacceptable risk for AEs.

8. Subject was being treated with botulinum toxin (BT)–containing regimens, plans to initiate such regimens during the DB or blinded follow-up portions of the study, or received surgical or BT treatment for spasticity-related complications within the 16 weeks prior to the first dose of study treatment in this study.

9. Was currently participating in another therapeutic clinical study or had received any investigational agent within 30 days (or 5 half-lives whichever is longer) prior to the first dose of study treatment in this study.

10. Previous liver or hematopoietic transplant procedure.

- **Treatments**

All patients started with a 0.1 mg/kg dose (or placebo-equivalent), which could be titrated with steps of 0.5 mg/kg, within a range of 0.05-0.2 mg/kg in the blinded study phase, guided by the plasma arginine levels.

Dose Modification

The dose was modified if the subject's plasma arginine level – assessed 168 hours after a given dose but prior to the next dose – is outside the range of 50 to 150 µM. Beginning with Visit 5 and ending with Visit LTE24, dose modifications, if required, based on plasma arginine values were implemented by the unblinded pharmacist and/or physician according to the following algorithm that will be implemented in the IXRS:

• If the plasma arginine level is >150 µM, a single 168-hour sample will be used to increase the dose by 2 dose levels (not to exceed 0.20 mg/kg) if the 2 doses prior to this sample were a) the same dose level in mg/kg, and b) consecutive (with no missed doses).

• If the plasma arginine levels from 2 sequential 168-hour samples (regardless of missed doses) are both <50 µM, the dose is decreased by 1 dose level to a minimum of 0.05 mg/kg.

Table 6 Dose adjustments for pegzilarginase

Dose Level[a]	Dose
1 (Minimum Possible Dose)	0.05 mg/kg
2 (Starting Dose)	0.10 mg/kg
3	0.15 mg/kg
4 (Maximum Possible Dose)	0.20 mg/kg

[a] Pegzilarginase dosing starts at level 2, 0.10 mg/kg. Dose increases, when required, are by 2 dose levels. Dose decreases are by 1 dose level.

Dosing in the extension phase
Subjects initially randomised to pegzilarginase received the former optimised dose they received during the 24-week DB period. Subjects initially randomised to placebo during the 24-week DB period began the 8-week DB period at a dose of 0.10 mg/kg that was permitted to be adjusted during the LTE period based on arginine levels.

Switching to subcutaneous (SC) dosing
After the first 8 weeks of the blinded LTE period, subjects had the option to receive pegzilarginase by SC administration, with Investigator and sponsor approval. The initial SC dose was the same as the IV dose unless otherwise dictated by arginine levels and could also be adjusted thereafter based on arginine levels.

The first 4 SC doses were given at the investigational site. Subsequent SC doses could be administered outside of the investigational site by appropriately trained home healthcare personnel if considered safe and appropriate in the opinion of the Investigator and Sponsor.

- **Objectives**

The primary objective is to demonstrate the efficacy of pegzilarginase relative to placebo based on a statistically significant decrease in plasma arginine levels.

The key secondary objective is to demonstrate the efficacy of pegzilarginase relative to placebo based on key mobility outcome measures.

- **Outcomes/endpoints**

The primary endpoint is change from baseline in plasma arginine after 24 weeks of study treatment.

There are 2 key secondary endpoints:

• Mean change from Baseline at Week 24 in the 2-Minute Walk Test (2MWT), and

• Mean change from Baseline at Week 24 in the Gross Motor Function Measure-88 Part E (GMFM-E).

The 2MWT is a performance outcome measure of mobility. Results for this test were recorded as distance walked during 2 minutes in meters. It was measured at screening, Week 12 and 24. The test was performed in patients above 3 years of age, as it cannot be reliably measured in the very young due to the natural motor development.

The Gross Motor Function Measure-88 (GMFM) is a clinical measure designed to evaluate gross motor function by observing the subject's ability to initiate and complete certain movements. GMFM-E is the subscale that measures the motor function in the domain of walking, running and jumping. Part E consists of 24 questions that will be used to calculate a Total Part E score (the final score is an integer between 0 and 72). Change from Baseline at Week 24 will be calculated for each measure as the Week 24 total score – the baseline score.

Secondary endpoints included:

• The proportion of subjects whose endpoint arginine value falls below the target from treatment guidance documents of 200 µM after 24 weeks of study treatment.

• The proportion of subjects whose endpoint arginine value falls within the normal reference range of $\geqslant$40 µM to $\leqslant$115 µM after 24 weeks of study treatment.

• Mean change from Baseline in ornithine and guanidino compounds (GC) after 24 weeks of study treatment.

• Mean change from Baseline at Week 24 in the GMFM-88 Part D (standing).

This domain of the GMFM-88 assesses standing. Gross Motor Function Measure D consists of 13 questions that will be used to calculate a Total Part D score (the final score is an integer between 0 and 39);

• Mean change from Baseline at Week 24 in the Functional Mobility Assessment (Functional Mobility Score (FMS) Measured at screening, Week 12 and 24.

• Mean change from Baseline at Week 24 in the Gillette Functional Assessment Questionnaire (GFAQ)) Measured at screening, Week 12 and 24.

• Mean change from Baseline at Week 24 in the VABS-II (Vineland Adaptive Behavior Scale, Second Edition) (Measured at BL, Week 12, 24).

Tertiary endpoints:

• proportions of responders in composite clinical outcome: a responder is defined as a subject exhibiting a mobility response in the 2MWT or GMFM-88 Part D or GMFM-88 Part E

A MCID response was pre-defined as

2MWT – ≥9% in distance walked (meters)

GMFM-D – ≥2.4 for GMFCS I at baseline, ≥3.3 for GMFCS II, and ≥1.5 for GMFCS III at baseline

GMFM-E – ≥4.0 for GMFCS I, ≥2.8 for GMFCS II, and ≥1.8 for GMFCS III

• improvement of spasticity (Modified Ashworth Scale, MAS).

• Caregiver/Clinician Global Impression of Change and Impression of Severity at Week 6, 12, 18, 24.

• change in fine motor function (9-Hole Pegboard) from the age of 3 years old) (screening, Week 12, 24).

• QoL (PedsQL for children 2-18 years, SF-36 (adult subjects).

• neurocognition and memory (BSID-III (Bayley Scales of Infant and Toddler Development, Third Edition, as age appropriate, age 2-3.5 years), Wechsler Intelligence Batteries (2.5-7.6 years, 6-16 years, >16 years).

• maintenance of diet (3-day diet records), BL, Week 6,12,18 and 24.

• growth and body weight (Z-scores for height, weight, and BMI were computed using the CDC growth curves for subjects younger than 18 years of age).

- **Sample size**

A total sample size of 30 subjects, with 20 subjects assigned to the pegzilarginase group and 10 subjects assigned to the placebo group and assuming a relative decrease of 77% from baseline in plasma arginine after 24 weekly doses of pegzilarginase with a common standard deviation 0.47 in ln-scale, alpha of 0.05 and the assumption that the change from baseline for placebo is 0, will lead to a power > 95%.

- **Randomisation and Blinding (masking)**

Central randomisation (2:1 ratio, pegzilarginase: placebo) was used to minimize bias. Randomisation was accomplished through IXRS. Randomisation was stratified by the severity of prior history of hyperammonaemia.

Investigators and patients/caretakers were blinded during the DB study phase. Blinding was continued during the first 8 weeks in the LTE study, where all patients received pegzilarginase, including those assigned to placebo in the initial DB study phase. Each site had an unblinded pharmacist and an unblinded physician to manage the protocol-defined dose adjustments. Laboratory results for arginine and ornithine have the potential of unblinding. Therefore, results for these laboratory tests performed during the 24 -week DB period will not be provided to the investigator or other blinded individuals.

- **Statistical methods**

Analysis sets

Randomised Set: Include all subjects in the consented set who are randomised to a blinded study treatment.

Full Analysis Set: Include all subjects who are randomised and who received at least 1 dose of blinded study treatment.

Post-hoc revised definition of the *Per-Protocol (PP) set*: Include all subjects in the FAS who had adequate exposure without important protocol deviations that directly impacted efficacy analyses.

Multiplicity

To control the overall type I error, hierarchical testing will be used, including the Hochberg procedure for testing the two key secondary endpoints and sequential testing of the other secondary endpoints.

Baseline Handling for Study Pauses and Re-Starts Due to COVID-19 during the DB period

- Subjects who paused during screening due to COVID-19 were allowed to restart the study. At the study restart, the site continued screening the subject and repeating assessments as necessary. Previous baseline assessments were reused (e.g., medical history) or updated if necessary.

- Subjects who paused during the DB period due to COVID-19 were allowed to re-start the study in accordance with the Study Guidance for COVID-19 Subject Handling Document. Subjects who had received fewer than 6 doses of study drug were re-started from Dose 1. Subjects who had received 6 or more doses were to be handled on an individual basis. At study restart, the site repeated baseline assessments in up to 3 further visits. Previous baseline assessments were reused (e.g., medical history) or updated if necessary.

Primary efficacy endpoint and efficacy analysis method

The primary and key secondary analyses were performed for the FAS and PP population.

The *primary endpoint* is the change from Baseline in plasma arginine after 24 weeks of study treatment.

Baseline plasma arginine value is the logarithmic value of the mean plasma arginine concentrations obtained during the Screening/Baseline Period and prior to the first dose of blinded study treatment.

The *final follow-up arginine value for 24 weeks* (endpoint arginine value) is the mean of the last 4 prior-to-dosing log-transformed values obtained during the 24-week DB period that meet the following criteria: 1. The sample date occurred after the scheduled date for the 20th dose; 2. The prior 2 doses were administered as planned (i.e., at approximately 7 days and 14 days prior to collection of the 24-week 168-hour arginine sample). Note: If at least 1, but fewer than 4, values meet the criteria, then only those values will be included in the mean. If none of the values meet the criteria, then the last single post-baseline arginine value will be included. If no post-baseline arginine values were obtained, then the change from Baseline will be imputed as 0.

The *primary efficacy analysis* is based on the change from baseline in the log-scale using an ANCOVA / MMRM model, including treatment, visit, treatment-by-visit interaction as fixed factors and baseline as covariate. Results per treatment group will be presented as geometric mean, geometric least squared (GLS) mean ratio for change (week 24/ Baseline), and Percent reduction in GLS mean at week 24 compared to baseline with 95% CIs. The treatment effect will be presented as a Percent reduction in GLS mean ratio for change (treatment/placebo) with 95% CIs and a 2-sided p-value.

If a final value (week 24) is unavailable, the change from Baseline was imputed by using the LOCF method (post-hoc decision). A treatment policy strategy was used for the intercurrent events discontinue due to AEs and more than 15% change in protein-restricted diet.

The Wilcoxon-rank-sum test and the analysis based on the ANCOVA/MMRM model using raw data (no log- transformation) are presented as *sensitivity analyses.*

Key secondary endpoints and analyses

• Mean change from Baseline at Week 24 in the 2-Minute Walk Test (2MWT), and

• Mean change from Baseline at Week 24 in the Gross Motor Function Measure-88 Part E (GMFM-E).

The key secondary endpoints are analysed using the MMRM model, including treatment, visit, treatment-by-visit interaction, and the baseline value as a covariate.

Secondary and tertiary endpoints and analyses

All secondary and tertiary analyses will be performed on the FAS only.

- For all responder analyses, a 2-sided Fisher's exact test was used. If data is missing at the timepoint of interest, the subject is considered a non-responder for the purpose of the analysis. Responder analysis was performed for the endpoints arginine value is <200 µM and arginine value falls within 40 to 115 µM.

- Change from Baseline in GCs and ornithine after 24 weeks of study treatment was analysed and summarised on logged data using the same methods as described for the primary endpoint (MMRM and missing final values imputed by LOCF value).

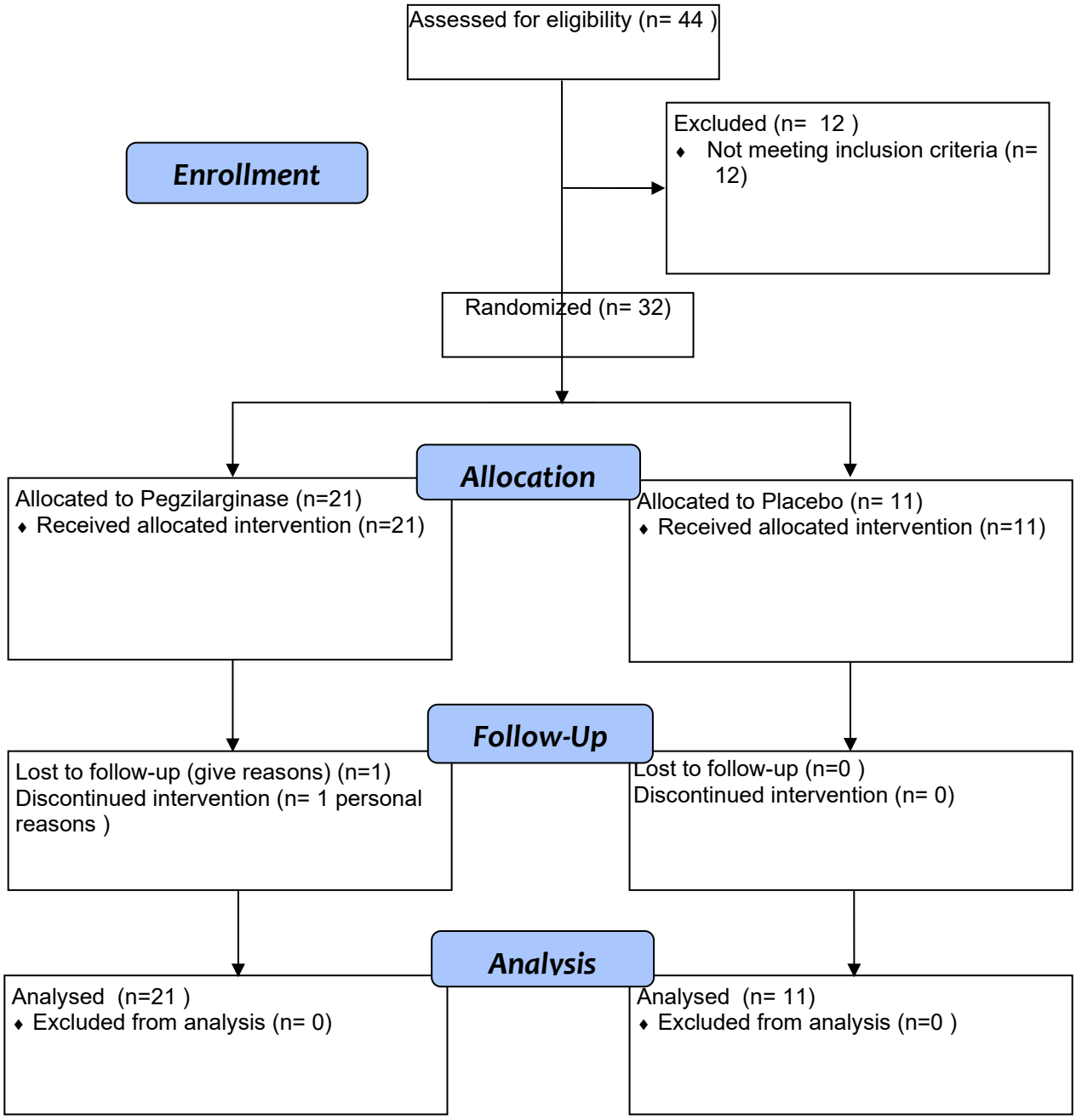
- Mean change from Baseline at Week 24 was analysed and summarised using the same methods as the key secondary endpoints (MMRM and no imputation for missing values) for the parameters GMFM-D, FMS 5, FMS 50, FMS 500, GFAQ, VABS-II Adaptive Behaviour Composite, VABS-II Communication Domain, VABS-II Daily Living Skills Domain, VABS-II Socialization Domain, VABS-II Motor Skills Domain.

Results

- **Participant flow**

As of the data cut-off date (14 October 2021), 44 subjects had been screened for the study. Of these 44 subjects who consented, 12 [27.3%] were screen failures, and 32 were eligible and were randomised in a 2:1 ratio to either the pegzilarginase (N=21) group or the placebo (N=11) group. Thirty-one out of 32 randomised subjects completed the DB period; 1 subject in the pegzilarginase treatment group discontinued the study what was reported to be due to personal reasons on Day 36. All 31 subjects who completed the DB period continued in the LTE part of the study.

Figure 10 Disposition of subjects in Study 300A



- **Recruitment**

First subject enrolled: 01 May 2019

Data cut-off date: 14 October 2021

Database lock DB period: 19 Nov 2021

Release date of report: 21 February 2022

Recruitment is complete. Also, the initial DB 24 weeks period is completed. However, the LTE part of the study (up to approximately 150 weeks) is still on-going.

- **Conduct of the study**

This study was initiated at 23 study sites, 19 of which enrolled and treated subjects. Nine sites were in the US, 4 in the UK, 1 in Austria, 1 in Canada, 2 sites in France, 1 in Germany, and 1 in Italy.

Protocol deviations

As of the data cut-off date, 81.3% of subjects experienced at least 1 important protocol deviation; of these, 76.2% (N=16) in the pegzilarginase group and 90.9% (N=10) in the placebo group experienced at least 1 important protocol deviation (Table 16).

In the double-blind period, 8 subjects (25%, 6 vs 2 in Pegzilarginase and Placebo group, respectively) had incorrect dosing during the treatment period due to IWRS issues.

Influence of the COVID-19 pandemic

As the general measures for public health and patients' care varied among the counties/continents where the study took place, no specific amendments were made in the Study protocol for enrolment and continuation. A total of 11 subjects (34.4%) experienced important protocol deviations that were COVID-19 pandemic related. For five subjects, the screening period of the study was extended to 137-274 days. In the DB period, 9.5% (2 subjects) in the pegzilarginase group and 9.1% (1 subject) in the placebo group had pauses ranging from 155 to 231 days in the study. In the LTE period, 3.2% (1 subject) had a pause in the study that was 211 days (Table 7).

Table 7 Summary of important protocol deviations by category (full analysis set)

Site Category	Pegzilarginase (N=21) n (%)	Placebo (N=11) n (%)	Overall (N=32) n (%)
Subjects with at least 1 important protocol deviation	16 (76.2)	10 (90.9)	26 (81.3)
COVID-19 deviations	5 (23.8)	6 (54.5)	11 (34.4)
IP compliance	1 (4.8)	0	1 (3.1)
Informed consent	1 (4.8)	0	1 (3.1)
Missed neuro assessment	0	1 (9.1)	1 (3.1)
Screening pause	2 (9.5)	2 (18.2)	4 (12.5)
Study procedures criteria	0	1 (9.1)	1 (3.1)
Study suspension	2 (9.5)	1 (9.1)	3 (9.4)
Visit schedule criteria	1 (4.8)	2 (18.2)	3 (9.4)
Non-COVID-19 deviations	16 (76.2)	9 (81.8)	25 (78.1)
Administrative criteria	1 (4.8)	0	1 (3.1)
IP Compliance	8 (38.1)	3 (27.3)	11 (34.4)
Informed Consent	5 (23.8)	4 (36.4)	9 (28.1)

Site Category	Pegzilarginase (N=21) n (%)	Placebo (N=11) n (%)	Overall (N=32) n (%)
Laboratory assessment criteria	2 (9.5)	3 (27.3)	5 (15.6)
RA or CEC approvals criteria	1 (4.8)	0	1 (3.1)
SAE criteria	0	1 (9.1)	1 (3.1)
Source document criteria	3 (14.3)	2 (18.2)	5 (15.6)
Study procedures criteria	11 (52.4)	6 (54.5)	17 (53.1)
Visit schedule criteria	2 (9.5)	0	2 (6.3)

Abbreviations: CEC=Central Ethics Committee; FAS=Full Analysis Set; IP=investigational product; N=number of subjects; RA=Regulatory Authority; SAE=serious adverse event. Note: Each subject was counted only once for each applicable specific violation/deviation. Percentages were based on the number of subjects in the FAS.

Treatment compliance, as defined by correctly receiving the intended dose, was ensured by site personnel with IP administration. Subjects were assessed for treatment compliance. The median treatment compliance was 95.8% for the pegzilarginase group and 100.0% for the placebo group in the DB period.

- **Baseline data**

Baseline demographics

The mean age of all subjects was 10.7 years (range: 2 to 29 years, Table 8). The mean age in the pegzilarginase group was younger (9.6, range: 2 to 28) compared to the placebo group (12.9, range: 5 to 29). The proportion of subjects in the age category of 2 to <6 years was higher in the pegzilarginase group versus the placebo group, with age categories of 6 to <12 and 12 to <18 years being relatively balanced in both treatment groups. One subject in the pegzilarginase group and 2 subjects in the placebo group were adults.

The other demographic characteristics of both groups were comparable.

Table 8 Baseline demographics Study 300A (full analysis set)

	Pegzilarginase (N=21)	Placebo (N=11)	Overall (N=32)
Age, years			
Mean (SD)	9.6 (6.16)	12.9 (6.77)	10.7 (6.47)
Median	8.0	12.0	10.5
Minimum, maximum	2, 28	5, 29	2, 29
Age categories (years), n (%)			
2 - <6	5 (23.8)	1 (9.1)	6 (18.8)
6 - <12	8 (38.1)	4 (36.4)	12 (37.5)
12 - <18	7 (33.3)	4 (36.4)	11 (34.4)
≥18	1 (4.8)	2 (18.2)	3 (9.4)
Weight, kg			

Mean (SD)	29.2 (13.55)	41.3 (17.90)	33.4 (16.00)
Median	27.1	36.2	31.0
Minimum, maximum	12, 62	19, 76	12, 76
Height, cm			
Mean (SD)	124.0 (23.13)	138.8 (20.30)	129.1 (23.00)
Median	128.8	137.9	133.3
Minimum, maximum	77, 162	113, 170	77, 170
BMI, kg/m^2			
Mean (SD)	18.0 (3.04)	20.3 (4.02)	18.8 (3.52)
Median	17.6	20.4	18.1
Minimum, maximum	14, 25	15, 28	14, 28
Sex, n (%)			
Female	9 (42.9)	4 (36.4)	13 (40.6)
Male	12 (57.1)	7 (63.6)	19 (59.4)
Race, n (%)[a]			
Asian	3 (14.3)	3 (27.3)	6 (18.8)
Black/African American	0	2 (18.2)	2 (6.3)
White	10 (47.6)	4 (36.4)	14 (43.8)
Other	6 (28.6)	0	6 (18.8)
Multiple Race	1 (4.8)	1 (9.1)	2 (6.3)
Missing	1 (4.8)	1 (9.1)	2 (6.3)
Region, n (%)			
US	8 (38.1)	6 (54.5)	14 (43.8)
Non-US	13 (61.9)	5 (45.5)	18 (56.3)

Abbreviations: BMI=body mass index; SD=standard deviation; US=United States.

Note: N in the headers represents the total number of subjects in the respective treatment group. Percentages are based on the total number of subjects in each respective treatment group. Age was calculated using the following equation: Age=(Informed Consent Date–Date of Birth)/365.25, truncated as an integer.

a Subjects who selected more than 1 race were included in the category Multiple Race.

Baseline clinical characteristics

The median age of diagnosis was 2.6 y (range 0-15). Twelve subjects (37.5%) required walking aids, including ankle-foot orthosis (n=7) and/or a wheelchair (n=6). The majority of subjects had difficulty walking a 'moderate' distance of about 50 m, encountered difficulties with climbing stairs, and had spasticity (21/32% for each disease characteristic). The study population was roughly equally distributed in patients with mild gross motor function deficiencies at baseline (GMFCS I, 43.8%), or moderate to severely affected patients (GMFCS II-IV, 56.2%). The majority had a cognitive delay (68.8). In addition, most had a history of LFT abnormalities (n=23, 71.9%, mostly transaminase increments), hyperammonaemia (n= 18, 56.3%), and/or seizures (n= 11 (34.4%)). See for details Table 9.

Table 9 Historical disease characteristics and baseline disease assessments (full analysis set)

	Pegzilarginase (N=21)	Placebo (N=11)	Overall (N=32)
Historical arginine level based on data obtained from medical records prior to enrollment (uM), means (SD)	409.4 (114.8)	476.1 (124.09)	433.87 (120.67)
Median	409.0	454.0	415.5
Minimum, maximum	173.0, 724.0	277.0, 664.0	173.0, 724.0
Age at initial symptoms, years			
Mean (SD)	1.6 (2.52)	2.5 (2.02)	1.9 (2.37)
Median	1.0	2.0	1.0
Minimum, maximum	0, 10	0, 7	0, 10
Age at diagnosis of ARG1 deficiency, (years)			
Mean (SD)	2.8 (4.06)	4.2 (3.07)	3.3 (3.75)
Median	0.7	4.6	2.6
Min, Max	0, 15	0, 11	0, 15
Walking ability - short distances, n (%)			
Normal	12 (57.1)	2 (18.2)	14 (43.8)
Minimal/moderate impairment	7 (33.3)	8 (72.7)	15 (46.9)
Severe impairment	2 (9.5)	1 (9.1)	3 (9.4)
Not able	0	0	0
Walking ability - moderate distances[b], n (%)			
Normal	9 (42.9)	2 (18.2)	11 (34.4)
Minimal/moderate impairment	8 (38.1)	6 (54.5)	14 (43.8)
Severe impairment	2 (9.5)	2 (18.2)	4 (12.5)
Not able	2 (9.5)	1 (9.1)	3 (9.4)
Walking ability - longer sustained distances[b], n (%)			
Normal	6 (28.6)	1 (9.1)	7 (21.9)
Minimal/moderate impairment	9 (42.9)	5 (45.5)	14 (43.8)
Severe impairment	4 (19.0)	1 (9.1)	5 (15.6)
Not able	2 (9.5)	4 (36.4)	6 (18.8)
Ability to climb stairs, n (%)			
Normal	9 (42.9)	2 (18.2)	11 (34.4)
Minimal/moderate impairment	6 (28.6)	5 (45.5)	11 (34.4)
Severe impairment	1 (4.8)	1 (9.1)	2 (6.3)
Not able	4 (19.0)	3 (27.3)	7 (21.9)

Does the subject require the use of an assisted device?, n (%)			
Yes	7 (33.3)	5 (45.5)	12 (37.5)
No	14 (66.7)	6 (54.5)	20 (62.5)
If Yes, Specify, n (%)			
Short distances	6 (28.6)	3 (27.3)	9 (28.1)
Moderate distances	5 (23.8)	5 (45.5)	10 (31.3)
Longer distances	6 (28.6)	4 (36.4)	10 (31.3)
What assisted device was used?[a], (n%)			
Ankle-foot orthosis	4 (19.0)	3 (27.3)	7 (21.9)
Single tip cane	1 (4.8)	0	1 (3.1)
Standard walker	1 (4.8)	1 (9.1)	2 (6.3)
Wheelchair	3 (14.3)	3 (27.3)	6 (18.8)
Other	1 (4.8)	1 (9.1)	2 (6.3)
GMFCS level at Baseline, n (%)			
I	9 (42.9)	5 (45.5)	14 (43.8)
II	9 (42.9)	4 (36.4)	13 (40.6)
III	0	0	0
IV	3 (14.3)	2 (18.2)	5 (15.6)
V	0	0	0
Level of spasticity, n (%)			
None	8 (38.1)	3 (27.3)	11 (34.4)
Mild	7 (33.3)	2 (18.2)	9 (28.1)
Moderate	5 (23.8)	4 (36.4)	9 (28.1)
Severe	1 (4.8)	2 (18.2)	3 (9.4)
muscle cramps?, n (%)			
Yes	3 (14.3)	4 (36.4)	7 (21.9)
No	18 (85.7)	7 (63.6)	25 (78.1)
seizures, n (%)			
Yes	7 (33.3)	4 (36.4)	11 (34.4)
No	14 (66.7)	7 (63.6)	21 (65.6)
Number of seizures in the last month			
Mean (SD)	0	0	0
Number of seizures in the last year			
Mean (SD)	0.1 (0.38)	17.5 (35.00)	6.5 (21.08)
Median	0.0	0.0	0.0
Minimum, maximum	0, 1	0, 70	0, 70
Cognitive delays, n (%)			

Yes	12 (57.1)	10 (90.9)	22 (68.8)
No	9 (42.9)	1 (9.1)	10 (31.3)
Age of first cognitive delay, years			
N	10	9	19
Mean (SD)	4.1 (2.96)	6.4 (4.39)	5.2 (3.79)
Median	3.5	6.0	5.0
Minimum, maximum	1, 11	1, 13	1, 13
Speech/language delay?, n (%)			
Yes	13 (61.9)	7 (63.6)	20 (62.5)
No	8 (38.1)	4 (36.4)	12 (37.5)
Liver test abnormalities, n (%)			
Yes	14 (66.7)	9 (81.8)	23 (71.9)
No	7 (33.3)	2 (18.2)	9 (28.1)
Liver test abnormality[a], n (%)			
ALT/AST elevation	13 (61.9)	9 (81.8)	22 (68.8)
Prothrombin time/INR elevation	8 (38.1)	3 (27.3)	11 (34.4)
Abnormal fibrinogen	0	0	0
GGT elevation	2 (9.5)	0	2 (6.3)
Other	1 (4.8)	0	1 (3.1)
History of **hyperammonaemia**			
Yes	12 (57.1)	6 (54.5)	18 (56.3)
No	9 (42.9)	5 (45.5)	14 (43.8)
Age of first hyperammonaemia episode, years			
n	12	6	18
Mean (SD)	5.3 (7.26)	4.7 (4.89)	5.1 (6.42)
Median	2.5	3.5	3.0
Minimum, maximum	0, 25	0, 14	0, 25

Abbreviations: ALT=alanine aminotransferase; ARG1=arginase 1; ARG1-D=arginase 1 deficiency; AST=aspartate aminotransferase; DNA=deoxyribonucleic acid; GGT=gamma-glutamyl transferase; GMFCS=gross motor function classification system; INR=international normalised ratio; RBC=red blood cell; SD=standard deviation.

[a] Subjects can choose more than one category.

[b] Distances: Short distance=at home (5 meters); Moderate distance=at school (50 meters); Longer sustained distance=shopping at store (500 meters)

Co-medication

Almost all subjects in the pegzilarginase-treated group (95.2%, n=20) and the placebo group (81.8%, n=9) received ammonia scavengers during the study. Other frequently used medications in both the pegzilarginase and placebo groups were Amino acids/carbohydrates/minerals/vitamins, combinations (14 (66.7%) and 7 (63.6), for pegzilarginase and placebo group, respectively) including EAA Formulas (6 (28.6) and 2 (18.2)). Other common medications were baclofen (4 (19.0) and 3 (27.3)), cetirizine

(6 (28.6%) and 5 (45.5%)), proton pump inhibitors (1 (4.8) and 5 (45.5)), and H2 receptor antagonists 2 (9.5) 3 (27.3).

- **Numbers analysed**

As of the data cut-off date (14 October 2021), 44 subjects had been screened for the study. Of these 44 subjects who consented, 32 were eligible (n=12 [27.3%] were screen failures) and were randomised in a 2:1 ratio to either the pegzilarginase (n=21) group or the placebo (n=11) group. Thirty-one out of 32 randomised subjects completed the DB period; One subject in the pegzilarginase treatment group discontinued the study for personal reasons. All 31 subjects who completed the DB period continued on to the LTE portion of the study. See Table 10 for the numbers analysed.

One subject (in the pegzilarginase group) did not receive the study drug at Visit 11 due to the subject being admitted to the hospital for an SAE. The subject remained off the study drug until the next dose on 04 May 2021 at Visit 18. This subject was excluded from the Per-Protocol set due to the extensive study drug noncompliance, which may have directly impacted the efficacy analysis.

Table 10 Disposition of subjects and analyses sets for the db period of study 300A (all subjects screened)

	Pegzilarginase n (%)	**Placebo n (%)**	**Overall n (%)**
Consented (screened) Set	-	-	44
Screen failures	-	-	12
Randomised Set	21 (100)	11 (100)	32 (100)
Full Analysis Set	21 (100)	11 (100)	32 (100)
Per-protocol Set	20 (95.2)	11 (100)	31 (96.9)
Double-blind randomisation period completion [a]			
Completed	20 (95.2)	11 (100)	31 (96.9)
Discontinued	1 (4.8)	0	1 (3.1)
Reason for discontinuation [b]			
Subject decision	1 (100)	0	1 (100)

Abbreviations: DB=double-blind; FAS=Full Analysis Set; ICF=informed consent form; LTE=long-term extension. Note: Percentages were based on the total number of subjects randomised in each treatment group. Consented Set included all subjects who signed an ICF. Randomised Set included all subjects in the Consented Set who were randomised to a blinded study treatment. Full Analysis Set included all subjects who were randomised and received at least 1 dose of blinded study treatment Per-Protocol Set included all subjects in the FAS who had adequate exposure without important protocol deviations that directly impacted efficacy analyses.

a Completers were defined as subjects who did not discontinue from study prior to LTE and therefore completed the 24-week DB randomisation period.

b Percentages were based on total number of discontinuations during DB randomisation period.

- **Outcomes and estimation**

At Week 24 in the pegzilarginase assignment group, 2 patients were at the down-titrated low dose step of 0.05 QW, 5 remained on the initial starting dose of 0.1 mg/kg QW, 2 subjects received the interim dose of 1.5 mg/kg, and 10 were up-titrated to the maximum allowed dose of 0.2 mg/kg.

Primary outcome; arginine levels at Week 24

The primary analysis used the MMRM method. Log-transformed plasma arginine data were used in the analysis. One subject dropped out prior to Week 24; the corresponding missed Week 24 data was imputed using the LOCF approach.

At Baseline, the arrhythmic mean (SD) plasma arginine levels were lower in the pegzilarginase group (354.0 µM [1.30 µM]) than in the placebo group (464.7 µM [1.21 µM]) (see Table 11 below).

For the plasma arginine levels at Week 24, the relative geometric least squares (GLS) mean ratio for change (pegzilarginase/placebo) was 0.233, 95% CI 0.165, 0.329 (p<0.0001). Thus, the primary objective was met. In other words, pegzilarginase demonstrated a 76.7% (95% CI 67.1, 83.5%) reduction in GLS mean ratio for change of plasma arginine at Week 24 from baseline relative to placebo. Treatment with pegzilarginase resulted in a statistically significant reduction (p<0.0001) in plasma arginine compared to placebo, which reached the optimal reduction at Week 8, which was maintained through the end of the DB phase of this study at Week 24 (see Figure 11 below). The primary analysis based on ANCOVA showed comparable results: 78.0% (95% CI 68.4, 84.7%) reduction in GLS mean ratio for change of plasma arginine at Week 24 from baseline relative to placebo.

Sensitivity analysis included Wilcoxon Rank Sum and MMRM with raw data. Both sensitivity and post-hoc PP analysis results were consistent with the primary analysis (p<0.0001).

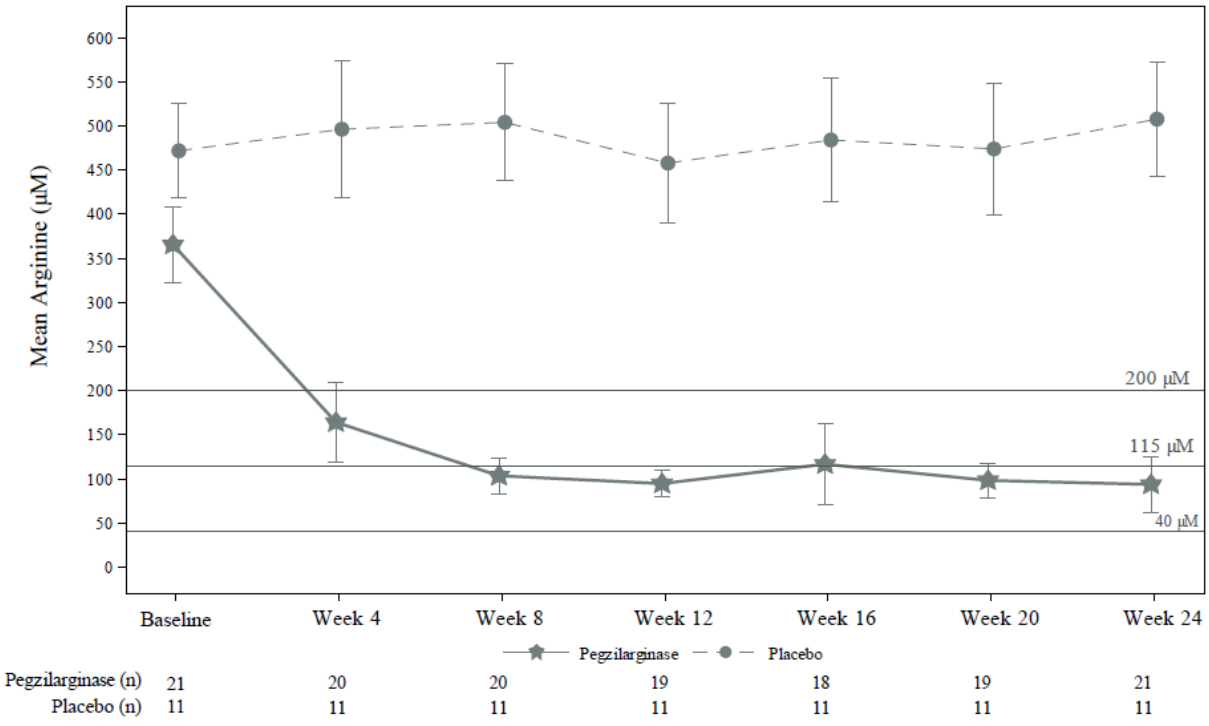
Table 11 Results of the primary efficacy analysis (MMRM analysis on log-transformed Arginine change from baseline to week 24) during the DB period (Full Analysis Set); Study 300A

Treatment	**N**	**Geom. mean at Baseline**	**Geom. mean at week 24**	**Geom. mean ratio for change (Week 24/ baseline)**	**GLS mean at week 24 [1]**	**GLS mean ratio for change [1] (week 24/ baseline) (95% CI)**	**Percent reduction in GLS mean at week 24 compared to baseline (%) [1] (95% CI)**
Pegzilarginase	21	354.0	86.4	0.244	90.7	0.233 (- 2.000, 2.467)	76.7 (-146.7,300.1)%
Placebo	11	464.7	426.5	0.918	389.6	1.000 (- 1.324, 3.324)	0.0 (-234.4,232.4)%

Treatment effect (Peg vs Placebo)		**p-value**
Relative GLS mean ratio for change	0.233 (0.165, 0.329)	<0.0001

(treatment/placebo) (95% CI) [1]		
Percent reduction in GLS mean ratio for change (treatment/placebo) (%) (95% CI) [1]	76.6 (67.1, 83.5)	
N=number of subjects included in the analysis CI= Confidence Interval Abbreviations: GLS = Geometric Least Squares; µM = Micromolar; MMRM = Mixed Model Repeated Measures;. [1]Based on a MMRM using log-transformed data with visit, study treatment, and interaction between visit and study treatment as fixed effects, and the logged baseline value included as a covariate. Default covariance structure type = unstructured.		

Figure 11 Summary of least square mean (95% CI) 168-hour post dose arginine levels (µM) over time in Study 300A double-blind period



Key secondary outcomes

GMFM-E

One subject in the pegzilarginase group did not have a Week 24 assessment because the subject withdrew from the study at Week 6 (no data imputation of missing data was planned for the secondary endpoints). Thus, 20 subjects in the pegzilarginase group were available for the analyses at Week 24.

The mean GMFM-E scores at baseline were similar for each assignment. The mean GMFM-E score in the pegzilarginase group increased by 4.2 points from Baseline to Week 24, compared to a decrease of

-0.4 points in the placebo group, resulting in an LS mean difference of 4.6 points at Week 24 (95% -1.1, 10.2, p=0.1087) (See Table 12).

Table 12 Summary of GMFM-E score improvement from baseline at week 24 during the DB period in Study 300A (full analysis set)

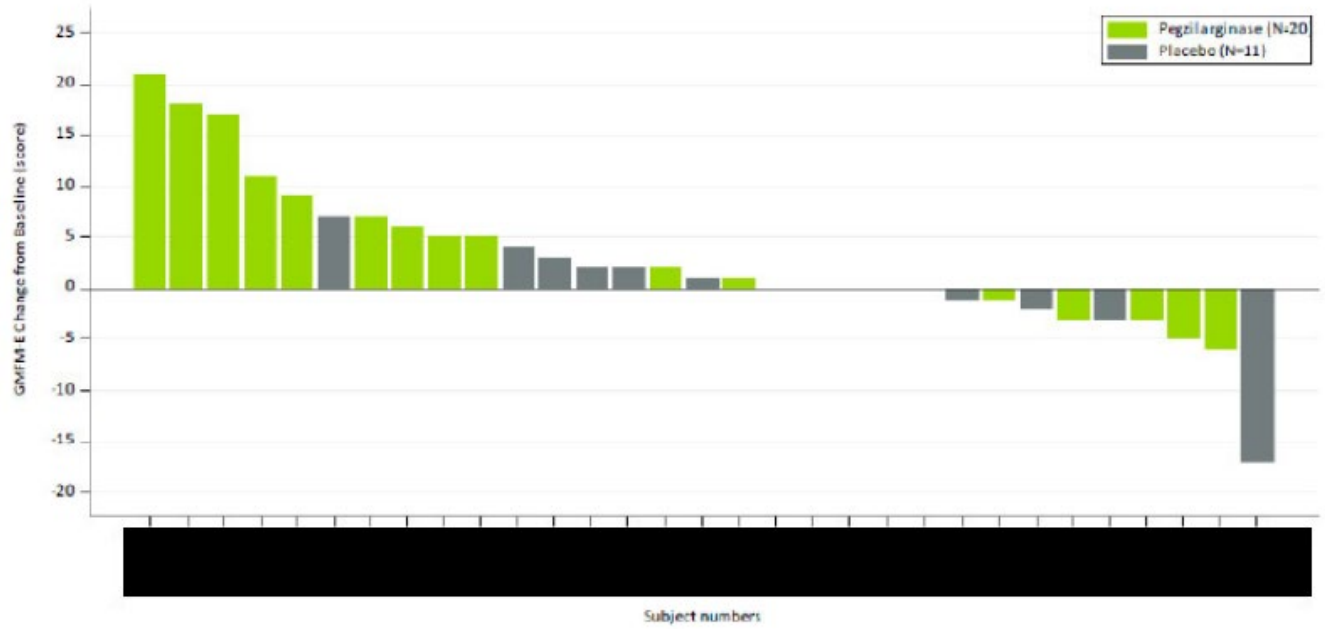
	Baseline		Week 24	
	Pegzilarginase (N=21)	**Placebo (N=11)**	**Pegzilarginase (N=20)**	**Placebo (N=11)**
Mean (SD)	48.3 (19.93)	46.5 (24.56)	52.0 (21.27)	46.1 (25.71)
Median	53.0	56.0	57.0	59.0
Min, max	5, 71	0, 72	2, 72	0, 71
LS Mean[1]			52.4	47.8
Change from Baseline to Week 24			N=20	N=10
LS Mean[1]			4.2	-0.4
LS Mean Diff. (Pegzilarginase–Placebo)[1]			4.6	
95% CI for LS Mean Diff.[1]			-1.1, 10.2	
p-value – MMRM[1]			0.1087	
p-value WRS[2]			0.3207	

1Based on an MMRM with visit, randomised study treatment, and interaction between visit and randomised study treatment as effects, and Baseline value included as a covariate. Default covariance structure type=unstructured. 2 Sensitivity analysis based on WRS test.

A post-hoc sensitivity assessment of GMFM-E excluding the subject with the incomplete baseline score showed a similar effect (LS Mean difference 4.8, 95% CI -1.1, 10.2)

A similar proportion of subjects in the pegzilarginase group (55.0%, n=11) had improvements in change from Baseline in GMFM-E compared to the placebo group (54.5%, n=6); however, the magnitude of improvement was generally larger in the pegzilarginase subjects compared to placebo subjects (see Waterfall plot below Figure 12).

Figure 12 Waterfall plot of GMFM-E change from baseline at week 24 during the DB period (full analysis set)



DB=double-blind; GMFM-E=Gross Motor Function Classification-88 Part E. Note: The waterfall plot includes all subjects including those missing assessments or with data inconsistencies.

2MWT

One subject in the Pegzilarginase group who completed the DB period had a missing Baseline assessment; because of their young age (<3 years old) at study entrance since the 2 MWT could not be reliably assessed in this age group (this was pre-defined in the protocol).

One subject in the placebo group did not have a Week 24 assessment because of pain in the knee, and one subject in the pegzilarginase group did not have a Week 24 assessment because they withdrew from the study at Week 6, and no data imputation of missing data was planned for the secondary endpoints according to the protocol. Furthermore, one subject in the placebo group was non-ambulatory at Baseline and wheelchair dependent.

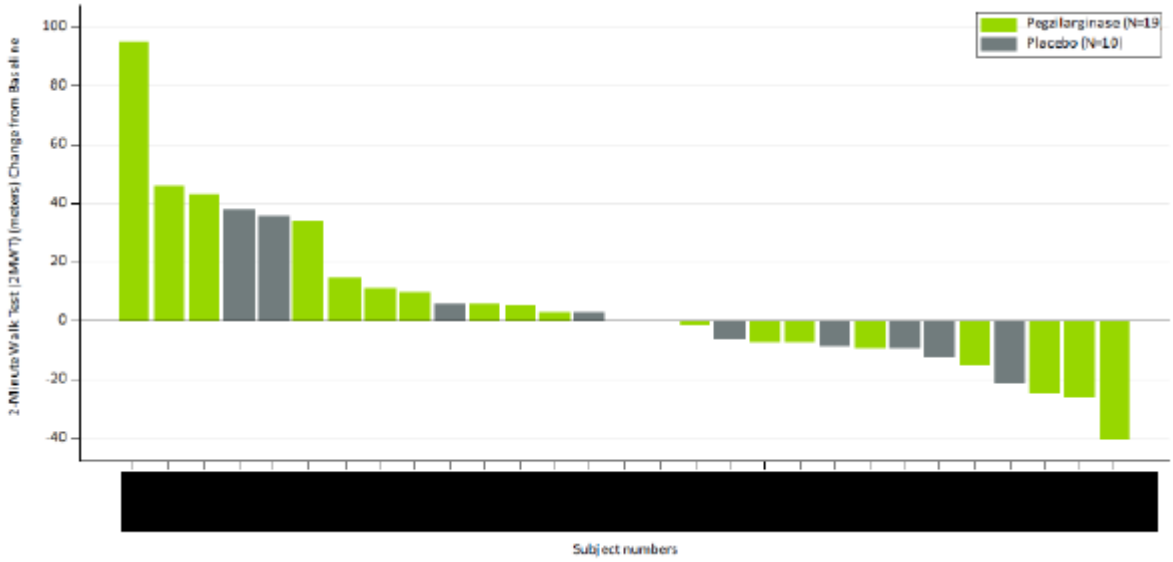
At baseline, the mean (SD) distance walked over 2 minutes for subjects who received pegzilarginase was longer at 109.0 meters (55.76 meters) compared to subjects who received placebo at 99.9 meters (49.00 meters). In addition, the mean distance walked over 2 minutes in the pegzilarginase group increased by 7.4 meters (19/21 subjects) from baseline to Week 24, compared to an increase of 1.9 meters (10/11 subjects) in the placebo group, leading to an LS mean difference of 5.5 meters (95% CI -15.6, 26.7, p=0.5961). See Table 13.

Table 13 Summary of 2MWT score improvement from baseline at week 24 during the DB period in Study 300A (full analysis set)

	Baseline		Week 24	
	Pegzilarginase (N=20)	**Placebo (N=11)**	**Pegzilarginase (N=20)**	**Placebo (N=10)**
Mean (SD)	109.0 (55.76)	99.9 (49.00)	115.9 (51.81)	102.3 (51.10)
Median	122.0	102.0	124.5	117.0
Min, max	2, 202	0, 171	1.0, 176.0	0, 163.0
LS Mean[1]			113.2	107.7
Change from Baseline to Week 24			**n=19**	**n=10**
LS Mean[1]			7.4	1.9
LS Mean Diff. (Pegzilarginase-–Placebo)[1]			5.5	-
95% CI for LS Mean Diff.[1]			-15.6, 26.7	-
p-value - MMRM[1]			0.5961	-
p-value WRS[2]			0.6599	-

1Based on an MMRM with visit, randomised study treatment, and interaction between visit and randomised study treatment as effects, and Baseline value included as a covariate. Default covariance structure type=unstructured. 2 Sensitivity analysis based on WRS test.

Figure 13 Waterfall plot of 2MWT change from baseline at week 24 during the DB period (full analysis set)



Secondary endpoints *(presented in order of the SAP)*

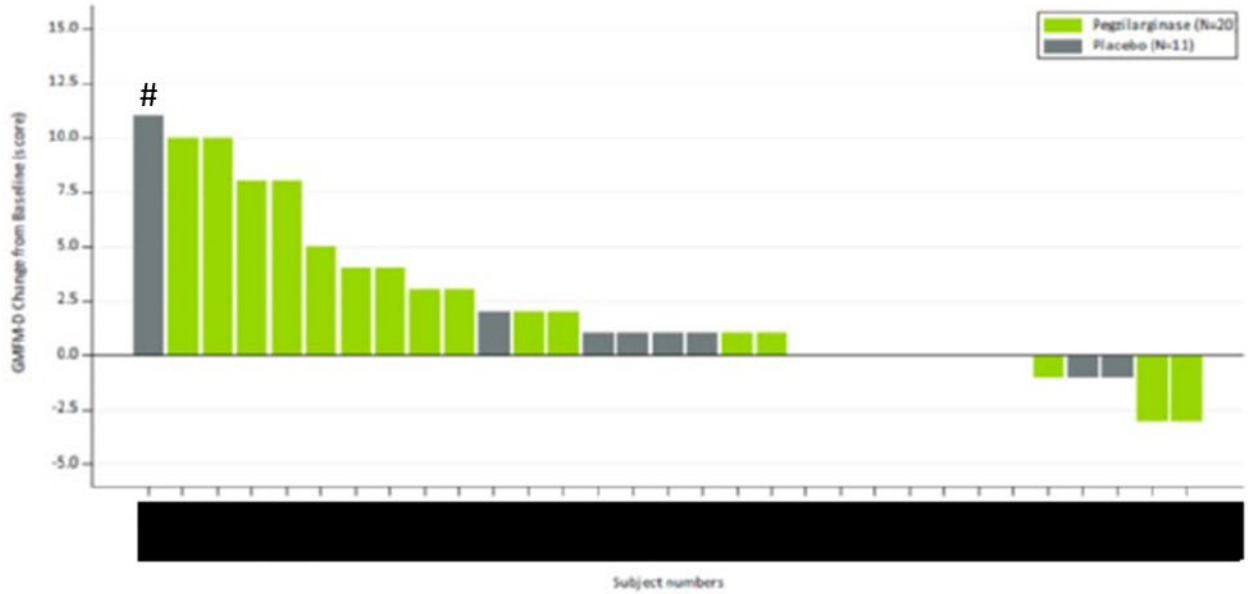
Responders based on arginine target levels

At Week 24, 90.5% (95% CI 71.5, 100%) of subjects in the pegzilarginase group met the criteria for a response for having achieved arginine values less than 200 µM, the treatment goal of plasma arginine according to treatment guidelines of ARG1-D. The response was the same for those having achieved arginine values within the normal range (≥40 µM to ≤115 µM). None of the subjects in the placebo group met either criterion for response.

GMFM-D (standing)

The mean GMFM-d scores at baseline were similar for each assignment group. For the FAS, the difference in the LS mean change from baseline was 1.4 (95% CI -1.4, 4.2). When post-hoc, an outlier was excluded from the placebo group who was not tested at Baseline and had erroneously a zero entered for the baseline score; the mean GMFM-D score in the pegzilarginase group increased by 2.7 points from Baseline to Week 24, compared to an increase of 0.4 points in the placebo group, resulting in a LS mean difference of 2.3 points (95% CI -0.4,4.9).

Figure 14 Waterfall plot of GMFM-D change from baseline at week 24 during the DB period (full analysis set)



Note: Subject was not tested at Baseline and had erroneously a zero entered for the baseline score.

Changes in Guanidino Compounds (GC) at Week 24

For the pegzilarginase group, decreases in the mean levels of all 4 GCs (ARGA (argininic acid), GAA (guanidinoacetic acid), GVA (alpha-keto-δ-guanidinovaleric acid), and NAArg (alpha-N-acetylarginine)) continued over time through Week 24 of the DB period. At Week 24, pegzilarginase demonstrated a 50% to 70% reduction in the 4 GCs compared to placebo (geometric mean ratio). Whereas for the placebo group, levels of all 4 GCs fluctuated over time but remained similar to Baseline levels through Week 24.

Tertiary outcomes

MCID Responder analyses for motor function outcomes

As per protocol, subjects classified as GMFCS-IV at baseline were excluded as there was no published MCID for a response. One subject was excluded as he withdrew from the study and had no post-Baseline assessments. At Week 24, the proportion of responders based on eligible subjects (n=17 for pegzilarginase and n=9 for placebo) was numerically higher for the pegzilarginase group for each individual component (2MWT, GMFM-D, and GMFM-E). Subjects achieving ≥2 or more responder thresholds were only found in the pegzilarginase group (6/17), and not for placebo.

Diet

A higher proportion of subjects in the pegzilarginase group (38.1%) than in the placebo group (18.2%) consumed >15% of total calories per day compared to Baseline. Similar was noted for total consumed protein (including natural and EAA protein) were 42.9% in the pegzilarginase group compared to 18.2% in the placebo group consumed >15% of total protein compared to Baseline.

Clinician and Caregiver Global Impressions of Change Assessments did not indicate a difference versus placebo.

Paediatric QoL scores indicated a small numerical change from baseline in favour of perzilarginase.

- **Ancillary analyses**

Subgroup analyses (Study 300A)

Subgroup analyses were pre-scheduled on the primary analysis of change from Baseline in plasma arginine and the key secondary analyses of change from Baseline in 2MWT and GMFM-E if numbers within the subgroups were at least 4. The subgroups included the following:

• Age (<18 years old at Screening, ≥18years old at Screening);

• Sex (male, female);

• Region (US, non-US);

• Gross motor function classification system (GMFCS) classification at Baseline (I, ≥II).

Subgroup analyses for the primary outcome (arginine levels)

Only one adult patient was assigned to pegzilarginase, and two adults to placebo, thus, no meaningful subgroup analyses could be performed for these age groups. For information, the single adult who was assigned to pegzilarginase., had a baseline value of 508.3 uM, which dropped to 78.0 mM at Week 24. The two subjects that received placebo, had a geometric mean (SD) baseline level of 500.1 (1.05), and 497.2 (1.03) at Week 24.

There were no relevant differences for the primary outcome in the subgroup analyses based on sex, region and GMFCS classification.

Subgroup analyses for the key-secondary endpoints

Overall, the subgroup analyses based on sex do not indicate a significant influence of these covariates on the outcomes. However, there was a tendency that treatment response in the two key-secondary outcomes, 2 MWT and GMFM-E, was more pronounced in those patients with poorer motor function at baseline, as compared to the patients who were mildly impaired at baseline regarding motor function (Figures 20 and 21). The applicant explained that this was probably due to the fact that patients with GMFCS class I at baseline are often at or near the top score of motor function, thus providing limited room for improvement (ceiling effect).

For 2 MWT there was a tendency for a larger treatment effect in the US than in non-US patients, although for the other key secondary endpoint GMFM-E, there was no apparent regional effect.

Region:

Mean (SD) baseline scores for US subjects were 49.8 (18.92) for pegzilarginase and 48.0 (25.50) for placebo. At Week 24, mean (SD) change from Baseline increased by 5.6 (7.41) and 2.2 (3.19) in the

pegzilarginase and placebo-treated groups, respectively. The LS mean difference (CI) between treatment groups numerically favoured pegzilarginase by 3.6 points (-3.7, 10.9).

Mean (SD) baseline scores for non-US subjects were 47.5 (21.24) for pegzilarginase and 44.6 (26.22) for placebo. At Week 24, mean (SD) change from Baseline increased by 3.3 (8.05) in the pegzilarginase group and decreased by 3.4 (7.89) in the placebo group. The LS mean difference (CI) between treatment groups numerically favoured pegzilarginase by 6.7 points (-2.8, 16.1).

Figure 15 Mean 2MWT score change from baseline in Study 300A; subgroups based on GMFCS at baseline

(I, ≥II)

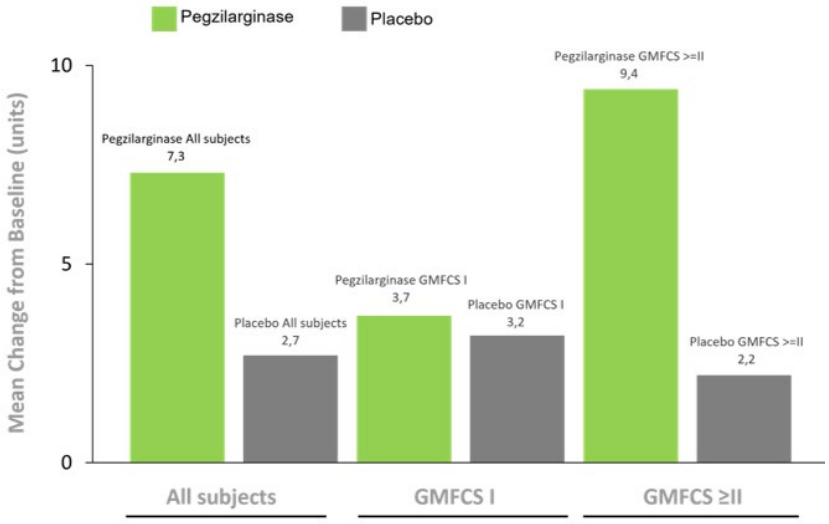
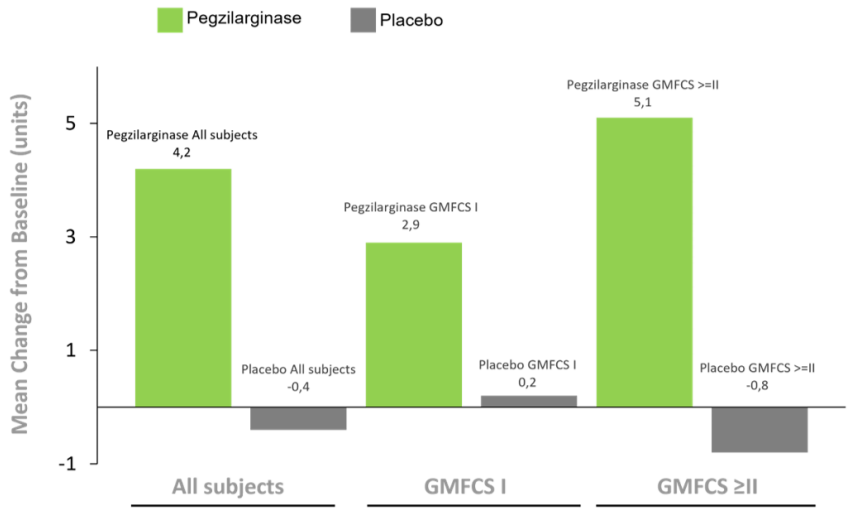


Figure 16 Mean GMFM-E score change from baseline in Study 300A; subgroups based on GMFCS) classification at baseline (I, ≥II)



Abbreviations: GMFM-E=Gross Motor Function Classification Item E (walking, running, and jumping).

Summary of main efficacy results

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

Table 14 Summary of efficacy for trial 300A

Title: PEACE (Pegzilarginase Effect on Arginase 1 Deficiency Clinical Endpoints): A Randomized, Double Blind, Placebo-Controlled Phase 3 Study of the Efficacy and Safety of Pegzilarginase in Children and Adults with Arginase 1 Deficiency			
Study identifier	CAEB1102-300A; EudraCT number: 2018-004837-34; Clinicaltrials.gov identifier: NCT03921541		
Design	Study 300A is an ongoing, Phase 3, randomised, double-blind (DB), placebo-controlled, international, multi-centre study that includes a 24-week, DB, randomised, parallel treatment period followed by an open-label LTE period of approximately 150 weeks. Eligible subjects with ARG1-D were randomised 2:1 with stratification factor severity of prior history of hyperammonaemia, to receive weekly intravenous (IV) pegzilarginase or placebo during the 24-week DB treatment period. Following completion of randomised treatment, all subjects could enter the LTE period to receive pegzilarginase IV, while remaining blinded to previous treatment, for 8 weeks, followed by open-label pegzilarginase treatment with the option of subcutaneous (SC) administration at the same dose as given IV after visit LTE08 (at Week 8)		
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:		24 weeks Not applicable Up to 150 weeks
Hypothesis	Superiority		
Treatments groups	Pegzilarginase		Weekly IV infusions for 24 weeks, on top of individualised disease management (IDM) (n=21). Starting dose 0.10 mg/kg which could be modified, based on plasma arginine values, between 0.05 mg/kg to 0.20 mg/kg.
	Placebo		Weekly IV infusions of placebo on top of IDM, 24 weeks (n=11)
Endpoints and definitions	Primary endpoint	Plasma arginine	Change from Baseline in plasma arginine after 24 weeks of blinded study drug
	Key secondary endpoint	2MWT	Mean change from Baseline at Week 24 in the Two-Minute Walk Test The 2MWT measures the distance a subject can walk on a flat surface in 2 minutes as measured in meters; increases in meters indicate improvement
	Key secondary endpoint	GMFM-E	Mean change from Baseline at Week 24 in the GMFM-88 Part E

			The Gross Motor Function Measure Item E (GMFM-E) evaluates the subject's ability to walk, run, and jump; higher scores indicate greater degree of ability, and increasing scores indicate that the subject show improvement.
	Secondary endpoint	Plasma arginine target responders	Arginine Values below the clinically recommended target level (<200 µM) at Week 24 (Number of Subjects, n/N)
	Secondary endpoint	arginine levels within normal range responders	Arginine Values within normal reference level (40-115 µM) at Week 24 (Number of Subjects, n/N)
	Secondary endpoint	GMFM-D	Mean change from Baseline at Week 24 in the GMFM-88 Part D The Gross Motor Function Measure Item D (GMFM-D) evaluates the subject's ability to stand; higher scores indicate greater ability, with increasing scores indicating improvement
Database lock	19 November 2021 14 October 2021:DB-period completed, and data cut-off date for available long-term extension data)		

Results and Analysis

Analysis description	**Primary Analysis**		
Analysis population and time point description	Full Analysis Set: All subjects who were randomised and received a least 1 dose of blinded study treatment. All safety and efficacy analyses were performed on this set. Time point: Week 24		
Descriptive statistics	Treatment group	Pegzilarginase	Placebo
	Number of subjects	21	11
	Plasma arginine, GLS Mean ratio for change (week 24/baseline) (95% CI) $	0.233 (-2.000, 2.467)	1.000 (-1.324, 3.324)
	Percent reduction in GLS mean at week 24 compared to baseline (%) (95% CI) (%)	76.7 (-146.7, 300.1)	0.0 (-234.4, 232.4)

	$		
	Number of subjects available for 2MWT assessment**	**19**	**10**
	2MWT, LS Mean change from BL score (SD)$	7.3 (30.64)	2.7 (19.66)
	Number of subjects GMFM-D (and E)***	**20 (20)**	**11 (10)**
	GMFM-E, LS Mean change from BL score (SD)$	4.2 (7.69)	-0.4 (6.20)
	Plasma arginine target (<200 µM)responders n/N (%) (95% CI)%	19 /21 (90.5) (71.5, 100)	0/11 (0) (0, 28.5)
	arginine levels within normal range (40-115µM) responders n/N (%) (95% CI)%	19 /21 (90.5) (71.5, 100)	0/11 (0) (0, 28.5)
	GMFM-D, Mean change from BL score (SD)$	2.7 (3.88)	0.4 (0.97)
Effect estimate per comparison	Primary endpoint Plasma arginine	Comparison groups	Pegzilarginase vs Placebo
		Percent reduction in GLS mean ratio for (treatment/placebo) (%)	76.6%
		95% Confidence Interval	67.1%, 83.5%
		P-value (Mixed Model Repeated Measures)	<0.0001
	Key secondary 2MWT	Comparison groups	Pegzilarginase vs Placebo
		Estimated Least Squares Mean Difference (Pegzilarginase- Placebo)	5.5
		95% Confidence Interval	-15.6, 26.7
		P-value (Mixed Model Repeated Measures)	0.60
	Key secondary GMFM-E	Comparison groups	Pegzilarginase vs Placebo
		Estimated Least Squares Mean Difference (Pegzilarginase- Placebo)	4.6
		95% Confidence Interval	-1.1, 10.2
		P-value (Mixed Model Repeated Measures)	0.1087
	Plasma arginine target responders (n/N)#	Comparison groups	Pegzilarginase vs Placebo
		Difference in proportions (%)	90.5
		95% Confidence Interval (%)	(58.7, 98.8)

	arginine levels within normal range responders#	Comparison groups	Pegzilarginase vs Placebo
		Difference in proportions	90.5
		95% Confidence Interval (%)	(58.7, 98.8)
	Secondary GMFM-D#	Comparison groups	Pegzilarginase vs Placebo
		Estimated Least Squares Mean Difference (Pegzilarginase-– Placebo)	2.3
		95% Confidence Interval	-0.4, 4.9
Notes	One subject in the pegzilarginase group discontinued the study for personal reasons at Week 6. For the primary analysis (plasma arginine levels), the corresponding missed Week 24 data were imputed using the LOCF approach. **not all subjects could perform the 2MWT as they were non-ambulatory at BL, or too young (< age of 3 years) *** One subject was excluded from the pegzilarginase group due to study withdrawal as by protocol it was decided not to impute missing data for (key secondary endpoint. For GMFM-D, one subject was excluded post-hoc from the placebo group due to erroneous baseline value (0 for missing data). #The analyses of the secondary endpoint are exploratory according to the pre-planned testing strategy as the key secondary endpoints failed to meet statistical significancy. $ 95% CI were not reported.		
Analysis description	**primary Analysis** The primary efficacy analysis is based on log-transformed arginine levels using the MMRM model with visit, treatment, and interaction between visit and treatment as fixed effects, and Baseline value included as a covariate. Sensitivity analysis included Wilcoxon Rank Sum and MMRM with raw data (not log-transformed). Both sensitivity analysis results were consistent with the primary analysis (p<0.0001). One subject dropped out after 6 weeks of treatment for "personal reasons " (not treatment related). LOCF was used as imputation.		
	Key secondary analyses; endpoints were analysed using an MMRM model. The treatment effect was presented as a difference in change from Baseline with a 95% CI (97.5% if only one of the 2 key secondary endpoints is significant from the Hochberg procedure) and a 2-sided p-value. The MMRM included treatment, visit, treatment-by-visit interaction, and the baseline value as a covariate. **Secondary analyses;** These could not be formally tested according to the pre-planned testing strategy, since the key secondary endpoints failed to meet statistical significance.		

2.6.5.3. Clinical studies in special populations

Not applicable

2.6.5.4. In vitro biomarker test for patient selection for efficacy

Not applicable

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

A heatmap of MCID responders was presented (Figure 17), summarising the results from Study 300A and 102A, and the preliminary data from the on-going long-term extension studies.

The heat map only included evaluable subjects and not the FAS. The reasons were that there are no MCID response thresholds available from the literature for patients with GMFCS Level IV at baseline, i.e. those with severe motor impairment. Therefore, those subjects were excluded from these analyses (Study 300A: 3 subjects in the pegzilarginase group, and 2 in the placebo arm Study 102A: one subject), as were those without a post-Baseline assessment at the relevant time point.

In the pegzilarginase group of the randomised study 300A, 6/17 evaluable subjects had MCID improvements at 2-3 multiple motor function domains. Incidental MCID responses were also observed in 4 subjects of the placebo group. However, these only occurred for a single motor function domain in the individual placebo responders and never for multiple ones.

In the long-term extension phase of the studies, the clinical responses continued to improve. As can be read from the heatmap analyses and Table 15.

Table 15 Subjects with response on 2 components without worsening

	Week 24		LTE 24	LTE 48	LTE 72	LTE120
Pegzilarginase	6/17	Pegzilarginase-Pegzilarginase	9/16	10/16	12/17	10/12
Placebo	0/9	Placebo-Pegzilarginase	1/7	0/8	1/8	2/5

Figure 17 Heat map of responders in Studies 300A and 102A and the extension studies (evaluable subjects)

Green: exceeded the MCID criterion; Red: met MCID criterion for worsening; White: did not meet MCID criterion for response or worsening; Blue: achieved maximum GMFM-D or E score; Grey: assessments were not performed ; Turquoise hash: achieved maximum score on GMFM-D or E or age/sex-matched normal walk distance and met MCID criterion

Abbreviations: 2MWT = 2-minute walk test [Study 300A]; 6MWT = 6-minute walk test [Study 102A]; GMFCS = Gross Motor Function Classification System; GMFM-D = Gross Motor Function Measure Item D (standing); GMFM-E = Gross Motor Function Measure Item E (walking, running and jumping); MCID = Minimal Clinically Important Difference; LTE = Long-Term Extension.

Five patients from Study 300A with the most severe impairment at baseline (GMFCS level IV) were necessarily excluded from these analyses, as no MCID value has been defined in the literature for this severity level. However, there are indications that also patients in the most severe disease stage GMFCS IV at baseline improved: Three of five subjects improved to level III at their last assessment. Level III means that they still need walking aids but without the need of continuous assistance of caretakers at mobility (level IV). For these 3 subjects, this was accompanied by an increase in 2MWT distance and GMFM-D score that exceeded the MCID thresholds.

2.6.5.6. Supportive study

Open-label extension study 102A (Study code CAEB1102-102A; EudraCT number: 2018-003163-67; Clinicaltrials.gov identifier: NCT03378531)

Design, conduct, endpoints

Study 102A is an open-label, uncontrolled international, multicentre study to evaluate the long-term safety, tolerability, and efficacy of pegzilarginase in patients with arginase 1 deficiency (ARG1-D). It is the extension of Study 101A (see under section dose-finding studies). Its primary objective is to evaluate the long-term safety and tolerability of IV or SC pegzilarginase administered for up to 4 years.

The study is on-going (interim study report data cut-off date: 11 June 2021) and the final study report will be provided in the post-marketing setting. Data are available for up to 120 weeks for individual patients. In this report, data of outcomes are presented of Week 48, i.e., the last timepoint with clinical data for all 13 subjects who continued the study.

Clinical outcomes included (amongst others) the 6MWT, GMFM-E and GMFM-D. Descriptive analyses were performed regarding the within-subject change from Baseline. Missing data were not imputed in the analyses.

Treatments in Study 102A

The dose was weekly intravenous (IV) infusions of pegzilarginase for Weeks 1 through 24, with the option of subcutaneous (SC) administration from Week 25 onward. The initial pegzilarginase dose level in Study 102A generally matched the dose the subject had last received at the end of Study 101A, but the dose could then be adjusted (to a maximum of 0.2 mg/kg) to maintain plasma arginine below the guideline-recommended level of 200 µM, and if achievable in the normal range (defined in the studies as 40 to 115 µM. The same dose as the last IV dose was used when switching to SC.

The background individualised disease management (IDM) was maintained, consistent with their disease management prior to the study.

Study population

At the start of Study 102A, the median age of subjects was 14.0 years (range: 6 to 32 years); 6 subjects were 2 to 11 years of age, 3 subjects were 12 to 17 years of age, and 5 subjects were $\geqslant$18 years of age. The majority of subjects were ambulatory (12/14, 85.7%) and classified as GMFCS Level I or II (11/14, 78.6%). Spasticity was common and reported in 10 of 14 subjects, with 7 subjects reported, and 6/14 subjects (42.9%) had a prior history of hyperammonaemia.

All 14 subjects that finished previous Study 101A and were eligible for the LTE study 102A were enrolled. One subject withdrew after 26 weeks of IV treatment in Study 102A, not deemed to be related to the study drug.

All remaining 13 subjects switched to SC administration. A total of 10/13 subjects received SC administration by home healthcare. In general, the subjects remained at the same dose as the previous IV dose; in 4 subjects, the dose was slightly increased, and in 2 subjects, the dose was reduced till Week 96.

Due to the COVID-19 pandemic, 8 subjects missed a single neurological/neuromotor function assessment, 3 subjects missed 2 function assessments, and 6 subjects had delayed neurological/neuromotor assessments. This mainly occurred after Week 48.

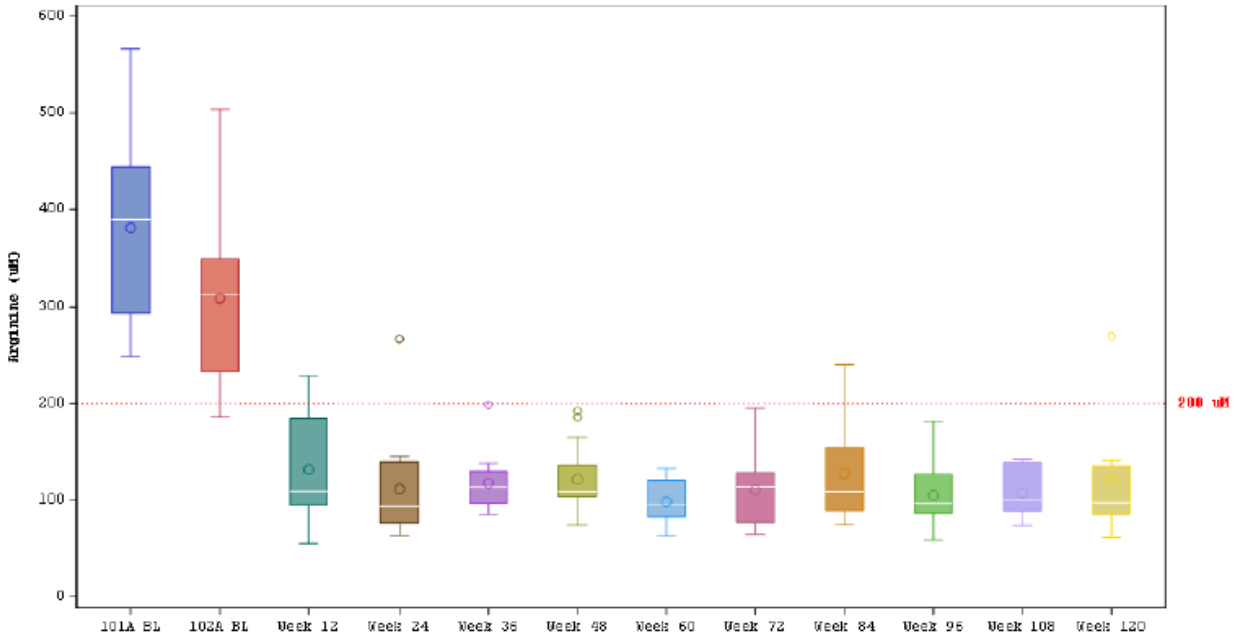
Arginine and guanidino levels

In general, arginine levels were maintained below the target level of 200 uM in this study. At Week 48, all subjects had achieved 168-hour post-dose plasma arginine levels (calculated from the last 3 samples) at the guideline-recommended level (<200 µM), and 9/13 subjects (69.2%) were within the normal range. No subjects had 168-hour post-dose plasma arginine levels <40 µM at 168 hours post-dose on Week 12- 120.

Anti-pegzilarginase antibodies were only detected in 1 subject at Baseline, were not detected at later time points in the study and had thus no notable impact on plasma arginine reduction.

GC compounds rapidly declined from baseline to Week 12, and remained continuously low thereafter. Plasma Argininic Acid (ARGA) declined form Baseline (Study 101A) by a median of -69%, Guanidinoacetic Acid (GAA) by -39%, α-keto-δ-guanidinovaleric Acid (GVA) by -58.5%, and α-N-acetylarginine (NAARG) by -69.7%.

Figure 18 Study 102A; box plot of plasma arginine level reduction at 168 hours post-dose (full analysis set)



Note: At week 96, n=13, At Week 120: n=6

Clinical outcomes

Motor function outcomes (GMFM-D, E and 6MWT) continued to improve from baseline of Study 101A to Week 48 (see Table 16).

Table 16 Long-term extension Study 102A; summary of the arginine motor function outcomes at Week 24 and 48, as compared to baseline (Study 101)

Outcome	**Study 101A Baseline** **Mean (SD)**	**102A; Week 24** Mean (SD)	LS mean differences from BL at Week 24	**102A** **Week 48** Mean (SD)	LS mean differences from BL (101A) at Week 48
	n=14	**n=14**	**n=14**	**n=13**	**n=13**
Plasma arginine (µM)	380.4 (89.71)	103.2 (73.68)	-277.2 (SD 124.27)	122.1 (52.2)	-267 (95% CI NR, SD 115)
GMFM-D	27.2 (11.60)	29.1 (11.0)	1.9 (0.9, 2.8) p<0.001	31.8 (8.40)	2.8 (95% CI 1.2, 4.3) , p <0.001
GMFM-E	44.8 (26.43)	48.9 (24.6)	4.1 (0.7, 7.6) p= 0.0199	53.6 (20.65)	5.8 (95% CI 2.5, 9.1), p <0.001
6MWT (meters)	301.2 (144.99)	322.6 (161.6)	21.3 (5.0, 59.5) p= 0.0212	346.2 (177.29)	44.9 (95% CI 18.6, 71.3), p = 0.0013

#Least Squares Means using Mixed Effect Model Repeated Measures

2.6.6. Discussion on clinical efficacy

Design and conduct of clinical studies

The exploratory dose-finding Study 101A, and its extension study 102A, was uncontrolled. However, this is considered adequate to establish the proof of concept of the drug, as this was based on a PD endpoint, arginine plasma levels, which is the target of the treatment. The interpretation of this biomarker endpoint is not biased by the open-label and uncontrolled design.

Design of the main study

The main study (300A) is a randomised, placebo-controlled trial, on top of previously used and unchanged standard of care with a protein-restricted diet (plus nitrogen scavengers if applicable). The choice of a placebo control is considered adequate, as no pharmacological treatments are available for this disease.

Pegzilarginase was administered in weekly IV infusions. The dose was adjusted based on the individual PD response of the arginine levels. The starting dose was 0.1 mg/kg in the DB period. Patients were transitioned from IV to SC administrations after the first 8 weeks of the LTE period.

ARG1-D was defined as having one of the following signs: elevated plasma arginine levels, a mutation analysis that resulted in a pathogenic variant, or reduced red blood cell arginase activity. Patients who had a hyperammonaemic episode (defined as an event in which a subject had an ammonia level ≥100 µM with 1 or more symptoms related to hyperammonaemia requiring hospitalisation or emergency room management) within the 6 weeks before the first dose of study treatment was administered were excluded from the study. This is acceptable.

The inclusion criteria of Study 300A, requiring a minimal baseline deficit of the key secondary clinical endpoints (2MWT and GMFM-D and -E) is understood to demonstrate clinical efficacy in a trial. However, from the exploratory Phase 1/2 study 101A and 102A, it was learnt that patients above or

near the ceiling of normality showed little room for improvement. Maintenance of normal motor function is also considered a legitimate treatment goal.

Only patients from the age of 2 years were eligible for the studies. This was in alignment of the PIP, which includes a deferral of patients younger than 2 years of age. The age limit ≥ 2 years has been adequately addressed in the SmPC wording of the indication.

Pegzilarginase was administered in weekly IV infusions. The dose was adjusted based on the individual PD response. The starting dose was 0.1 mg/kg in the DB period. Patients were transitioned from IV to SC administrations after the first 8 weeks of the LTE period. No dose adjustments are needed for this transition to SC dosing, as established on the arginine levels, which remained below target after this transition.

Stratification was based on a history of recent hyperammonaemia episodes in the last month before randomisation or two or more episodes in the previous year. This is understood as hyperammonaemia episodes may impact clinical outcomes, study treatment adherence and safety assessments.

Only a single randomised trial is performed (300A), which may be understood given the ultra-rarity of the disorder.

The double-blind placebo-controlled study phase of the main study was 24 weeks. After this, patients were eligible for the LTE phase, where all patients could receive pegzilarginase. The LTE study phase is still on-going and the final study report will be provided in the post-marketing setting. The duration of the 24 weeks placebo-control was discussed in the Scientific Advice. A longer period was considered more optimal, given the slow progression of the disease, and it may take time to achieve a change in clinical outcomes in this chronic neurodegenerative disorder. However, in the end, it was accepted for ethical reasons, and preliminary data from the Phase 1 study (101A) indicate that there were already changes in motor function at this short notice.

The blinding is maintained in the first 8 weeks of the LTE phase. The rationale for this is not fully understood, but no questions are raised on this, as it does not impact the assessments of the benefits.

Choice of the endpoints of the main study

Change in plasma arginine was selected as the primary endpoint as a) it is mechanistically related to the primary disorder, b) it is the single common manifestation in all subjects, c) it is believed to be the key driver of clinical manifestations and d) it can be objectively measured utilising a validated bioanalytical assay. Treatment guidelines emphasize the need to lower arginine levels in patients, even though this cannot be achieved with the current treatment option, i.e. dietary restriction. There are limited clinical data to support the recommended treatment target level of arginine < 200 uM, which is still above the upper normal level of 115 uM. In the SmPC, the treatment goal was set at <ULN, which is considered realistic given the high PD response of pegzilarginase in the clinical studies.

The applicant provided a comprehensive review of the literature, supporting the rationale for choosing plasma arginine as the primary surrogate endpoint. From the non-clinical *in-vitro* and *in-vivo* data, it has become clear that both arginine itself, as well as its toxic metabolites NO and GC (guanidino compounds), which are mainly formed in the liver, are potentially neurotoxic at systemic accumulation due to ARG1-D. Arginase-1 itself is lowly expressed in the brain in healthy humans. However, an excess of arginine and its toxic metabolites (guanidino compounds) has been demonstrated in the CSF in ARG1-D patients. There was a high correlation between plasma and liquor levels of these substances both in ARG1-D patients, illustrating their active distribution and passage through the BBB (blood-brain-barrier). Thus, although pegzilarginase itself does not pass the BBB, there is still a rationale to target peripheral arginine plasma levels. Indirect evidence of ERT by pegzilarginase was provided from other methods that restore arginase-1 deficiency, such as liver transplantation in patients and gene

replacement therapy in a relevant disease model (arginase-1 gene knock-out mice). In these studies, normalisation of the arginine plasma levels ameliorated neurological damage, as shown in MRI, histology and EEG, and clinical symptoms.

In cross-sectional or retrospective studies, the relationship between disease severity and arginine levels was unclear (Huemer, 2016). However, in a prospective natural history study (follow-up up to 7 years), one patient with arginine around the target level of 200 uM indeed developed, apart from aberrant behaviour, no motor function disorders. In contrast, the 5 other ARG1-D patients with hyperarginaemia all developed spasticity, with the severity of clinical symptoms related to their total accumulated arginine exposure.

The choice of arginine levels as a primary outcome was accepted in the SAWP/CHMP advice, although it was emphasised that the surrogacy should be supported by the clinical endpoints (preferably co-primary). The applicant did not follow the SA recommendations considering the upgrade of the key secondary outcome to a co-primary outcome. The applicant adapted the protocol to include motor function endpoint 2MWT and GMFM-E (gross motor function of domain walking, running, jumping) as key secondary. The choice of these motor function endpoints is supported. There are no specific instruments available for ARG1-D. The GMFM-88 scale was originally developed for children with cerebral palsy, but as ARG1-D patients have overlapping symptoms of spastic paraplegia, this scale is deemed acceptable.

Statistical methods

The study was powered based on the assumption of a relative decrease of 77% from baseline in plasma arginine after 24 weekly doses of pegzilarginase, and a common standard deviation of 0.681 in log2 scale [~0.47 in ln scale], both estimated from the Phase 1 /2 studies 101A and 102A. Together with 30 subjects, alpha of 0.05 and the assumption that the change from baseline for placebo is 0, will lead to a power > 95%. This is acceptable. Power calculation for the two co-key secondary endpoints is based on simulations, assuming effect sizes from studies 101A and 102A. The assumed correlations lack a justification, but these assumptions seem reasonable.

During the randomisation, the subjects were stratified by severity of prior history of hyperammonaemia. The stratification factor was not included in the primary efficacy analysis, which should have been. However, a post-hoc analysis including the stratification factor provided almost similar results.

The pre-planned primary efficacy analysis model was not clearly pre-specified. Therefore, both an ANCOVA model and an MMRM model were presented in SAP v3.0. The MMRM model was specified by presenting SAS codes. Post-hoc was decided to use the MMRM model as the primary efficacy analysis; results of the ANCOVA model led to the same conclusions. The analysis was to be performed on log-transformed data; however, for the average baseline value this was not calculated correctly. First, the average was calculated for four screening measurements, then log-transformed. For a geometric mean, the measurements should be log-transformed first and then averaged. The primary endpoint has been re-analysed accordingly, and there were no relevant differences from the previous primary analyses.

Post-hoc, an estimand was defined, describing a treatment policy strategy for the intercurrent events discontinued due to AE and more than 15% change in protein-restricted, which is acceptable.

The handling of missing final (week 24) arginine values changed post-hoc in using the LOCF method instead of imputing the pre-specified baseline value. This had no relevant impact on the outcomes.

Conduct

Overall, the number of protocol deviations also beyond the COVID-pandemic- are on the high side. The majority were related to study procedural criteria, which might be expected given the high number of measurements and the complexity of the dosing. The applicant discussed the deviations regarding the informed consent procedures, which were of an administrative nature or were related to a missing Spanish version of the informed consent form. The influence of IP interruption on the key secondary endpoints because of the pandemic was limited and balanced over the study arms.

Efficacy data and additional analyses

The proof of concept that pegzilarginase can reduce arginine levels has been adequately demonstrated in the Phase 1/2 study.

Study populations

Overall, the study populations could be considered representative for the target population regarding symptoms and disease severity. The mean baseline arginine levels were high. While approximately 50% of the subjects had relatively mild impairment at baseline, the other half had a moderate-severe motor impairment, with spasticity and often requiring walking aids.

Patients with a relatively modest level of hypoarginaemia (<250 uM), were excluded from the studies. According to the wording of the indication, these patients are eligible for treatment. Case reports of patients who achieve arginine levels near the target level of 200 uM with a diet alone describe that they did not develop motor impairment. However, based on the literature, it is known that the toxic GC metabolites may still accumulate above normal levels, even though the arginine levels could be reasonably controlled in some patients near the clinical target levels. Although there is a rationale to target the arginine levels within the physiological boundaries as toxicity may still occur due to long-circulating GC, the clinical benefits of Loargys in patients with arginine levels below or near the clinical threshold of 200 uM remain somewhat unclear since these patients were excluded from the trial, and their symptoms may be modest. The decision for treatment should be made on an individual basis and this was addressed in the SmPC section 4.4.

Relatively young patients were included in the studies (range 2-32 y median 10.5 y in Study 300A and 12 y in Study 101A). This is understood, given the shortened life expectancy of these patients, and early intervention is warranted in this progressive disease. However, middle-aged patients were described in the literature. Efficacy outcomes from adolescents and young adults to older patients may be difficult to extrapolate, as reversibility of clinical symptoms may be incomplete in older patients with long existing immobility and neurological damage. This uncertainty was addressed in the SmPC section 4.4.

Primary and key secondary outcomes

It has been confirmed that pegzilarginase could significantly reduce arginine levels in ARG1-D patients who had hyperarginaemia, despite a protein-restricted diet. Pegzilarginase demonstrated a 76.7% (95% CI 67.1, 83.5%) reduction in GLS mean ratio for change of plasma arginine at Week 24 from baseline relative to placebo. Illustrative of the magnitude of the primary outcomes is that the responder rates of subjects achieving arginine levels below the treatment goal of 200 µM, or 115uM, was 90.5% of subjects in the pegzilarginase group versus 0 in the placebo group.

However, the two clinical key secondary outcomes failed to meet their endpoints at a statistically significant level. Thus, their effect size after 24 weeks of treatment remains uncertain.

The study period of 24 weeks might be too short to obtain an optimal clinical effect in this chronic neurodegenerative disorder. It is considered promising that at continued treatment in the LTE studies, the MCID responder rates gradually continued to improve, also in patients switching from placebo. Also supportive is that in 3 out of the 5 most severe patients at baseline (GMFSM level IV), where no MCID has been defined in the literature as no improvement may be expected, improved at long-term follow-up in GMFM-D (standing) and 2MWT, and they did not require constant assistance of the caretaker anymore at mobility.

Consistently, in the long-term extension study 102A, where arginine plasma levels remained stable within the target levels, the motor function scores also improved from baseline at longer-term follow-up (48 weeks and longer). Although this is an uncontrolled study, the data could be considered supportive, as no improvement in GMFM scores might be expected given the progressive nature of the disease. Also, the 6MWT score increased, but this might be influenced by ageing. The GMFM scores are corrected for age.

Preliminary data from the two ongoing LTE studies showed that after approximately 2-3 years of follow-up, patients either improved in motor function, remained stable or achieved the normal function score for their age group (see heatmap table 19). Thus far, none of these patients who continued treatment in the LTE study worsened. The drop-out rate was low and not considered treatment-related.

After 24 weeks, the levels of the diverse GC were reduced by 50-70% from baseline, whereas these increased in the placebo control arm. According to some experts, these biomarkers are considered important drivers of neurotoxicity in ARG1-D patients -perhaps more important than arginine itself. Although reduced, the plasma levels of these longer circulating compounds were still above the upper level of the normal reference value, which might explain that even after 24 weeks the treatment effect may not be optimal. The applicant will provide the final study report on the GCs for CHMP assessment in the post-marketing setting.

There were imbalances at baseline, not in favour of the placebo group, with higher plasma arginine levels, higher age, higher rates of walking aids use and shorter 2MWD.

Imbalances at randomisation may occur in such small study populations. However, there was no clear relationship between age and GMFCS levels in the study population, a measure of the severity of motor function impairment. The MRMM analyses of the primary and key secondary endpoint corrected for differences at baseline. Reassuringly, the responses were higher in patients with a poor GMFCS score at baseline. Altogether, the imbalances at baseline are not expected to affect the primary and key secondary outcomes.

Dosing

Overall, the proposed dosing range is considered appropriate, given the high arginine response.

As discussed in the Safety section, IV doses lead to a prolonged period of hypoarginaemia, which is less likely to occur with SC administration. When using modelling and simulation, it became clear that in initial treatment with SC dosing instead of IV, adequate control of arginine was obtained. This has now been reflected in the SmPC posology section, which is supported, also given the convenience of the SC dose.

In the studies, specifically prepared tubes were used for blood sampling of the arginine plasma levels, with an enzyme blocker to inhibit residual pegzilarginase activity in the sample. In the SmPC, it has been added that validated measures should be used to monitor arginine levels, to obtain the correct

value for the dose determination. The applicant has committed that the prepared tubes with CE marks will become available for the clinics.

Supportive Study 102A

Reduction in plasma arginine and GC levels were observed in patients treated with pegzilarginase in long-term extension study 102A. At week 24 and Week 48, 92.9% and 100% of subjects achieved a plasma arginine level that met the guideline recommendations. 64.3% and 69.2% of subjects achieved arginine levels within the normal range, respectively.

Statistically significant from baseline was observed in GMFM-E with a mean change from study 101A Baseline of 7.1 (SD 7.52) at Week 96. Similar effects were shown for the other motor function outcomes. However, as no comparison to control group is possible, this data should be treated with caution.

Ancillary analyses

The finding that treatment effects of pegzilarginase regarding the MWTs and GMFM scales were larger in patients in GMFCS > II (i.e. patients with moderate-severe gross motor function impairment), than in patients with GMFCS I (i.e. patients with mild impairment), was also observed in Study 102A. As explained by the applicant, this may be related to a ceiling effect, as the patients with limited disability at baseline have baseline values near the top score, and there was little room of improvement. The fact that a higher response was shown in those patients with moderate-severe impairment may be considered as a support of (the relevance of) the treatment effect.

Assessment of paediatric data on clinical efficacy

The majority of the study data was obtained from paediatric and adolescent patients. Subgroup analyses were pre-scheduled with a cut-off of 18 years in the main Study 300A. As only three adult patients were included in this study, no meaningful subgroup analyses could be performed. Additional subgroup analyses are requested with other age cut-off values (6 and 12 y). Given the progressive nature of the disease these analyses may provide more insight in the effect in young children and adolescents.

Additional efficacy data needed in the context of a MA under exceptional circumstances

On the one hand, the primary endpoint of the reduction of arginine levels was met after 24 weeks of treatment, and the reduction of arginine by this ERT was durable for 2 years or more. On the other hand, some uncertainty remains regarding the precision of the effect size of the clinical endpoint of motor function in the randomised study since statistical significance was not met. The long-term extension studies showed promising clinical responses, also in severe patients, but the sample size is too small to conclude the effect size, durability of response and loss of response due to ADA formation. The adherence to the protein-restricted diet may decrease in daily practice, particularly once the arginine levels are stabilised. It is unclear whether dose increments could address this.

The posology in the SmPC is not strict regarding the upper dose, which is supported given the limited clinical experience thus far. The PK-PD modelling predicted that 3-5% of the patients might require a higher dose of 0.2 mg/kg as applied in the studies. Given these uncertainties, post-marketing information is needed regarding the adequacy of dosing.

Information about starting ERT with pegzilarginase in middle-aged and elderly adult patients is lacking. In addition, the reversibility of symptoms may be limited in adult patients with long-existing neurological damage and immobility due to spastic diplegia.

Altogether, the study data are not considered comprehensive, particularly as long-term efficacy and safety data are lacking, and there are some uncertainties regarding the optimal dose. Also, data in adult patients is limited. Given the ultra-rarity of the disease, it may be challenging to obtain long-term data.

SOB

Post-marketing efficacy data will be gathered in the context of an existing EU disease registry for metabolic disorders, called E-IMD. In this registry, data will be routinely monitored regarding the required pegzilarginase dose, arginine levels and dietary protein intake, and additionally, clinical outcomes on motor function and cognitive development.

In addition, the PASS will retrieve safety data from the existing E-IMD registry (see section on Clinical Safety below).

2.6.7. Conclusions on the clinical efficacy

Overall, it has been confirmed that pegzilarginase can effectively reduce arginine levels in patients with ARG1-D, below the clinical target levels and ULN.

It is considered that the clinical outcomes from the studies support the surrogacy of the primary PD endpoint.

However, the CHMP considers the following measures necessary to address the missing efficacy data in the context of a MA under exceptional circumstances (SOB):

- to fulfil the information gap on long-term maintenance of efficacy, data will be provided from the ongoing LTE studies. These will be category 2 post-authorisation measures.

- a registry-based efficacy study (E-IMD) will collect information on the long-term effectiveness/clinical outcomes in patients with ARG1-D treated with pegzilarginase. This will be a PAES, classified as a category 2 post-authorisation measure (SOB).

- in order to ensure adequate monitoring of safety and efficacy of pegzilarginase in the treatment of arginase 1 deficiency (ARG1-D) in adults, adolescents and children, the MAH shall provide yearly updates on any new information concerning the safety and efficacy of pegzilarginase. This will be a category 2 post-authorisation measure (SOB).

2.6.8. Clinical safety

2.6.8.1. Patient exposure

In total, 48 ARG1-D patients have been exposed to pegzilarginase in three studies.

The first 2 studies were open-label, multi-centre studies that were conducted serially in the same subjects: Study 101A (completed dose-finding study) and its long-term extension (LTE) study 102A

(ongoing). A total of 16 subjects aged 2 years and older with a documented diagnosis of ARG1-D were enrolled in Study 101A. Of these subjects, 14 subjects completed this study, and these were all enrolled in Study 102A.

The third study, Study 300A, is a Phase 3, multicentre, randomised, double-blind (DB), placebo-controlled study in subjects ≥2 years of age with ARG1-D designed to assess efficacy and safety. The study included a 24-week DB period, in which 32 subjects were randomised 2:1 to pegzilarginase or placebo respectively. Thirty-one subjects completed the 24-week DB period and entered an open-label LTE period of up to 150 weeks, where all subjects received pegzilarginase.

Across studies, there were equal numbers of males and females (n=24 each), and approximately half of the subjects were White (52.1%). The median age was 11.5 years (range 2 to 31 years). The majority of subjects had a history of liver function abnormalities (68.8%) and hyperammonaemia (52.1%). LFT abnormalities were mainly ALT/AST elevations (31/48, 64.6%). About 30% (15/48) of the subjects had a previous history of epilepsy at baseline.

At data cut-off, the pooled dataset of 48 subjects treated with pegzilarginase had a combined total of 3440 person-weeks of pegzilarginase exposure. Over half of the subjects (n=28) were treated with pegzilarginase for >1 year, and over one-fourth of subjects were treated with pegzilarginase for >2 years (maximum of 3.8 years) (see Table 17 below). The mean duration of exposure was 29.0 weeks for IV administration and 60.2 weeks for SC administration. While 23/48 (48%) subjects were titrated to the highest IV doses (>1.5-0.2 mg/kg), 12 subjects (25%) remained on the intermediate-dose level (0.1-<0.15 mg/kg), and 13 subjects (27%) were treated with low IV doses (<0.1 mg/kg). A similar distribution of dose levels was observed for the 36 subjects that received SC doses (see Table 18).

The following Safety datasets were used to analyze the data:

- Placebo-controlled, DB period of Study 300A of 24 weeks (n=21 for pegzilarginase, n=11 for placebo);

- All Pegzilarginase Treated population (n=48), which includes data from all subjects who received at least 1 dose of pegzilarginase in Studies 101A, 102A, or 300A (pooled analyses);

- Long-term safety dataset followed in the LTE parts of Study 102A (n=14) and Study 300A (n=31) is as per the cutoff dates.

Table 17 Summary of duration of exposure to pegzilarginase in the clinical study programme

		DB Period 300A		Any Pegzilarginase			
		Pegzilarginase N=21	**Placebo N=11**	**Study 101A N=16**	**Study 102A N=14**	**Study 300A LTE N=31**	**All Pegzilarginase Treated N=48**
Number of Subjects Exposed	N	21	11	16	14	31	48
Duration of Exposure (weeks)	Mean (SD)	23.1 (3.94)	23.8 (0.75)	13.1 (3.70)	127.6 (37.08)	31.1 (26.42)	71.7 (55.03)
	Median	24.0	24.0	14.0	130.5	31.0	58.5
	Min, Max	6, 25	22, 25	5, 17	26, 183	1, 102	1, 197
Total person-weeks		486.0	262.0	209.0	1786.0	963.0	3440.0
Treatment Duration Category, n (%)	≤4 weeks	0	0	0	0	--	3 (6.3)
	>4 to ≤24 weeks	20 (95.2)	10 (90.9)	16 (100)	0	--	5 (10.4)
	>24 to ≤52 weeks	1 (4.8)	1 (9.1)	0	1 (7.1)	--	12 (25.0)
	>52 to ≤104 weeks	0	0	0	0	--	15 (31.3)
	>104 weeks	0	0	0	13 (92.9)	--	13 (27.1)
	<24 weeks[a]	--	--	--	--	13 (41.9)	--
	≥24 weeks[a]	--	--	--	--	18 (58.1)	--

- Abbreviations: DB=double-blind; LTE=long-term extension; Max=maximum; Min=minimum; SD=standard deviation
- a For Study 300A LTE only

Table 18 Dose of exposure and treatment duration per route of administration

Dose of Exposure[a]	**Number of Subjects (N)**			**Person-weeks[b]**		
	IV	**SC**	**Total**	**IV**	**SC**	**Total**
<0.05 mg/kg	2	0	2	11.0	0	11.0
0.05 - <0.10 mg/kg	11	9	11	413.0	883.0	1296.0
0.10 - <0.15 mg/kg	12	9	12	359.0	495.0	855.0
0.15 - <0.20 mg/kg	23	16	23	609.0	668.0	1278.0
Total	48	34	48	1392.0	2046.0	3440.0

- Abbreviations: IV=intravenous; SC=subcutaneous
- a Dose of exposure is calculated as the mean dose over the whole study (pooling IV and SC doses) for each subject.
- b Person time is calculated by summing the total exposure time for subjects within each subgroup.

2.6.8.2. Adverse events

An independent review of the safety data was conducted by a Data Safety Monitoring Board in Studies 101A and 102A and a Safety Review Committee in Study 300A.

The vast majority of patients encountered one or more AEs. None of these led to dose reductions or withdrawal from the study. Temporary dose interruptions with pegzilarginase were common: 47.9% of the subjects (pooled dataset) had one or more interruptions because of TEAEs. Moreover, these occurred more frequently than placebo in the controlled study phase (Table 19 below).

The incidence of TEAEs overall was higher with IV administration than SC administration (35/48, 72.9% versus 20/34, 58.8%).

Safety by System Organ Class

As shown in Table 29, the most frequently reported TEAEs by *System Organ Class* which were more commonly reported for pegzilarginase than placebo in Study 300A were Infections and infestations (8 (38.1%) vs 1 (9.1%)), General disorders and administration site conditions (7 (33.3%) vs none), Injury, poisoning and procedural complications 5 (23.8%) vs none for placebo, and Eye disorders (4 (19%) vs none).

In the domain of General disorders and administration site conditions, pyrexia was reported in 4 subjects (19.0%). The majority of the Pyrexia TEAEs coincided with other events like infections. Furthermore, swelling face, peripheral swelling, oedema peripheral and administration site extravasation were reported as single TEAEs with pegzilarginase. The events were mild in all but the oedema peripheral case which was moderate. These events could be considered as infusion reactions.

In the DB period of Study 300A, 8 subjects in the pegzilarginase arm (38.1%) reported 12 TEAEs in the SOC Infections and infestations compared to 1 subject (9.1%) in the placebo arm. These were mainly nasopharyngitis, upper respiratory tract infections, pharyngitis and influenza, and single cases of respiratory syncytial virus infection, conjunctivitis, fungal skin infection, and gastrointestinal viral infection. The TEAEs were mild or moderate, all occurring in subjects younger than 18 years of age. None of the TEAEs was assessed as related to the treatment with pegzilarginase by the Investigator.

Other domains, such as gastrointestinal disorders, investigations, metabolism and nutrition disorders, were also common but equally distributed over the two assignment arms in Study 300A. These AEs were mainly related to nausea and vomiting, increased ammonia and hyperammonaemia and LFT increments, which are well-known disease characteristics.

Adverse events by Preferred terms

The most frequently reported TEAEs in PT terms in the pegzilarginase arm in Study 300A were vomiting (28.6%), pyrexia (19.0%), cough (19.0%), constipation (14.3%) and ammonia increased (14.3%). For the placebo, nausea and vomiting, hyperammonaemia and ammonia increased, and abdominal pain there the most frequent TEAE (Table 20).

Dose-response relationship

There were no meaningful dose-response relationships regarding the number or nature of adverse events. LFT increment and hyperammonaemia were more commonly reported in the middle-dose group and less in the highest-dose group (Table 22).

Dose interruptions due to AEs

In Study 300A, more subjects in the pegzilarginase arm than in the placebo arm had TEAEs that led to dose interruption (38.1% versus 9.1%). The TEAEs that led to dose interruption in more than 1 subject

in the pegzilarginase arm included pyrexia (3 subjects, 14.3%, all mild in severity), AST increased (2 subjects, 9.5%, all mild in severity), and hyperammonaemia (2 subjects moderate in 1 subject and severe in 1 subject). For the majority of subjects, the events resolved within 8 days and for all subjects, dosing was resumed.

Table 19 Overview of treatment-emergent adverse events

	DB Period: 300A		101A (N=16)	Long-term Extensions[a]		All Pegzilarginase Treated (N=48)
	Pegzilarginase (N=21)	Placebo (N=11)		Study 102A (N=14)	Study 300A LTE (N=31)	
	Subjects[b] n (%)	Subjects[b] n (%)	Subjects[b] n (%)	Subjects[b] n (%)	Subjects[b] n (%)	Subjects[b] n (%)
Any TEAE	18 (85.7)	11 (100)	16 (100)	14 (100)	23 (74.2)	44 (91.7)
Mild	10 (47.6)	5 (45.5)	4 (25.0)	1 (7.1)	11 (35.5)	14 (29.2)
Moderate	7 (33.3)	6 (54.5)	10 (62.5)	11 (78.6)	10 (32.3)	23 (47.9)
Severe	1 (4.8)	0	2 (12.5)	2 (14.3)	2 (6.5)	7 (14.6)
Related TEAE[c]	5 (23.8)	1 (9.1)	13 (81.3)	7 (50.0)	5 (16.1)	22 (45.8)
TEAE leading to dose reduction	0	0	0	0	0	0
TEAE leading to dose interruption	8 (38.1)	1 (9.1)	6 (37.5)	8 (57.1)	6 (19.4)	23 (47.9)
TEAE leading to discontinuation of study drug	0	0	0	0	0	0
Any TE SAE	4 (19.0)	4 (36.4)	8 (50.0)	8 (57.1)	6 (19.4)	19 (39.6)
Any Related TE SAE[d]	1 (4.8)	0	5 (31.3)	2 (14.3)	1 (3.2)	7 (14.6)
TEAE with fatal outcome	0	0	0	0	0	0

Abbreviations: AE=adverse event; DB=double-blind; FAS=full analysis set; LTE=long-term extension; MedDRA=Medical Dictionary for Regulatory Activities; SAE=serious adverse event; TE=treatment-emergent; TEAE=treatment-emergent adverse event.

Notes:

Percentages are based on the total number of subjects in the FAS. TEAEs were coded using MedDRA version 24.0

a One subject in the pegzilarginase arm in Study 300A withdrew from the study and did not enter the 300A LTE. Two subjects in Study 101A withdrew from the study and did not enter Study 102A.

b Subjects are only included in the category denoting the highest severity reported for all TEAEs on study.

c Relatedness as assessed by the Investigator.

Table 20 Safety by MeDRA system organ class

	DB Period: Study 300A		Study 101A (N=16)	Long-term Extensions		All Pegzilarginase Treated (N=48)
	Pegzilarginase (N=21)	Placebo (N=11)		Study 102A (N=14)	Study 300A LTE (N=31)	
System Organ Class	Subjects n (%)	Subjects n (%)	Subjects n (%)	Subjects n (%)	Subjects n (%)	Subjects n (%)
Any TEAE	18 (85.7)	11 (100)	16 (100)	14 (100)	23 (74.2)	44 (91.7)
Blood and lymphatic system disorders	2 (9.5)	0	1 (6.3)	1 (7.1)	1 (3.2)	5 (10.4)
Cardiac disorders	0	0	2 (12.5)	1 (7.1)	0	3 (6.3)
Ear and labyrinth disorders	0	0	2 (12.5)	2 (14.3)	1 (3.2)	4 (8.3)

Eye disorders	4 (19.0)	0	0	1 (7.1)	0	5 (10.4)
Gastrointestinal disorders	10 (47.6)	6 (54.5)	12 (75.0)	11 (78.6)	10 (32.3)	31 (64.6)
General disorders and administration site conditions	7 (33.3)	0	7 (43.8)	7 (50.0)	4 (12.9)	18 (37.5)
Hepatobiliary disorders	2 (9.5)	1 (9.1)	0	0	2 (6.5)	4 (8.3)
Immune system disorders	2 (9.5)	0	5 (31.3)	2 (14.3)	1 (3.2)	7 (14.6)
Infections and infestations	8 (38.1)	1 (9.1)	9 (56.3)	12 (85.7)	11 (35.5)	29 (60.4)
Injury, poisoning and procedural complications	5 (23.8)	0	2 (12.5)	5 (35.7)	4 (12.9)	15 (31.3)
Investigations	7 (33.3)	4 (36.4)	6 (37.5)	8 (57.1)	10 (32.3)	24 (50.0)
Metabolism and nutrition disorders	3 (14.3)	4 (36.4)	6 (37.5)	8 (57.1)	7 (22.6)	19 (39.6)
Musculoskeletal and connective tissue disorders	1 (4.8)	2 (18.2)	4 (25.0)	7 (50.0)	3 (9.7)	13 (27.1)
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	0	0	0	1 (7.1)	0	1 (2.1)
Nervous system disorders	5 (23.8)	3 (27.3)	5 (31.3)	7 (50.0)	5 (16.1)	17 (35.4)
Psychiatric disorders	2 (9.5)	2 (18.2)	3 (18.8)	4 (28.6)	0	8 (16.7)
Renal and urinary disorders	0	0	1 (6.3)	0	0	1 (2.1)
Reproductive system and breast disorders	0	0	1 (6.3)	1 (7.1)	0	1 (2.1)
Respiratory, thoracic and mediastinal disorders	5 (23.8)	2 (18.2)	6 (37.5)	8 (57.1)	5 (16.1)	17 (35.4)
Skin and subcutaneous tissue disorders	3 (14.3)	2 (18.2)	4 (25.0)	6 (42.9)	2 (6.5)	14 (29.2)
Vascular disorders	1 (4.8)	0	1 (6.3)	1 (7.1)	1 (3.2)	4 (8.3)

Abbreviations: AE=adverse event; DB=double-blind; FAS=full analysis set; LTE=long-term extension; MedDRA=Medical Dictionary for Regulatory Activities; SOC=system organ class; TEAE=treatment-emergent adverse event.

Notes: Percentages are based on the total number of subjects in the FAS. TEAEs were coded using the MedDRA version 24.0. SOCs are sorted alphabetically.

Table 21 Treatment-emergent adverse events occurring in ⩾10% of subjects in the all pegzilarginase treated population

	DB Period: Study 300A		**Study 101A (N=16)**	**Long-Term Extensions**		**All Pegzilarginase Treated (N=48)**
	Pegzilarginase (N=21)	**Placebo (N=11)**		**Study 102A (N=14)**	**Study 300A LTE (N=31)**	
Preferred Term	**Subjects n (%)**	**Subjects n (%)**	**Subjects n (%)**	**Subjects n (%)**	**Subjects n (%)**	**Subjects n (%)**
Vomiting	6 (28.6)	3 (27.3)	8 (50.0)	8 (57.1)	8 (25.8)	23 (47.9)
Cough	4 (19.0)	1 (9.1)	4 (25.0)	8 (57.1)	2 (6.5)	15 (31.3)
Headache	2 (9.5)	1 (9.1)	4 (25.0)	7 (50.0)	4 (12.9)	14 (29.2)
Hyperammonaemia	2 (9.5)	3 (27.3)	6 (37.5)	6 (42.9)	5 (16.1)	14 (29.2)
Ammonia increased	3 (14.3)	3 (27.3)	0	5 (35.7)	4 (12.9)	13 (27.1)
Nausea	1 (4.8)	4 (36.4)	3 (18.8)	6 (42.9)	5 (16.1)	12 (25.0)
Aspartate aminotransferase increased	2 (9.5)	0	3 (18.8)	2 (14.3)	5 (16.1)	11 (22.9)
Constipation	3 (14.3)	1 (9.1)	1 (6.3)	4 (28.6)	3 (9.7)	10 (20.8)

Alanine aminotransferase increased	2 (9.5)	0	3 (18.8)	1 (7.1)	5 (16.1)	9 (18.8)
Pyrexia	4 (19.0)	0	1 (6.3)	3 (21.4)	3 (9.7)	9 (18.8)
Upper respiratory tract infection	1 (4.8)	0	4 (25.0)	5 (35.7)	1 (3.2)	9 (18.8)
Abdominal pain	1 (4.8)	3 (27.3)	2 (12.5)	2 (14.3)	3 (9.7)	7 (14.6)
Abdominal pain upper	1 (4.8)	0	2 (12.5)	5 (35.7)	1 (3.2)	7 (14.6)
Diarrhoea	1 (4.8)	1 (9.1)	1 (6.3)	3 (21.4)	1 (3.2)	6 (12.5)
Nasopharyngitis	2 (9.5)	0	0	4 (28.6)	1 (3.2)	6 (12.5)
Amino acid level increased	1 (4.8)	0	0	1 (7.1)	3 (9.7)	5 (10.4)
Back pain	0	0	2 (12.5)	2 (14.3)	1 (3.2)	5 (10.4)
Gastroenteritis	0	0	1 (6.3)	3 (21.4)	2 (6.5)	5 (10.4)

Abbreviations: AE=adverse event; DB=double-blind; FAS=full analysis set; LTE=long-term extension; MedDRA= Medical Dictionary for Regulatory Activities; PT=preferred term

Notes: Percentages are based on the total number of subjects in the FAS. TEAEs were coded using MedDRA version 24.0. PTs are sorted by decreasingfrequency of All Pegzilarginase Treated; ties are sorted alphabetically within the All Pegzilarginase Treated column.

Table 22 Adverse events at different dose levels

	Dose <0.05mg/kg (N=1)		Dose 0.05- <0.10mg/kg (N=5)		Dose 0.10- <0.15mg/kg (N=9)		Dose 0.15- <0.2mg/kg (N=1)		Dose .2mg/kg (N=32)		All Pegzilarginase Treated (N=48)	
Preferred Term	Subjects n (%)	Events	Subjects n (%)	Events	Subjects n (%)	Events	Subjects n (%)	Events	Subjects n (%)	Events	Subjects n (%)	Events
Any Common TEAEs	0	0	5 (100)	17	8 (88.9)	163	1 (100)	1	24 (75.0)	166	38 (79.2)	347
Vomiting	0	0	1 (20.0)	1	7 (77.8)	33	0	0	15 (46.9)	30	23 (47.9)	64
Cough	0	0	1 (20.0)	1	5 (55.6)	14	1 (100)	1	8 (25.0)	19	15 (31.3)	35
Headache	0	0	2 (40.0)	3	5 (55.6)	24	0	0	7 (21.9)	9	14 (29.2)	36
Hyperammonaemia	0	0	1 (20.0)	1	5 (55.6)	40	0	0	8 (25.0)	18	14 (29.2)	59
Ammonia increased	0	0	0	0	6 (66.7)	9	0	0	7 (21.9)	12	13 (27.1)	21
Nausea	0	0	1 (20.0)	1	5 (55.6)	17	0	0	6 (18.8)	17	12 (25.0)	35
Aspartate aminotransferase increased	0	0	0	0	4 (44.4)	7	0	0	7 (21.9)	13	11 (22.9)	20
Constipation	0	0	1 (20.0)	1	2 (22.2)	3	0	0	7 (21.9)	13	10 (20.8)	17
Alanine aminotransferase increased	0	0	0	0	2 (22.2)	3	0	0	7 (21.9)	16	9 (18.8)	19
Pyrexia	0	0	1 (20.0)	1	2 (22.2)	4	0	0	6 (18.8)	7	9 (18.8)	12
Upper respiratory tract infection	0	0	2 (40.0)	2	3 (33.3)	9	0	0	4 (12.5)	6	9 (18.8)	17
Abdominal pain	0	0	2 (40.0)	6	0	0	0	0	6 (18.8)	6	8 (16.7)	12

Abbreviations: TEAE = Treatment Emergent Adverse Event.
Note: Column denominators are used to calculate percentage. The dose range group is assigned based on the maximum dose level administered to a subject during the study.
AEs are coded using Medical Dictionary for Regulatory Activities (MedDRA) version 24.0.
PTs are sorted by decreasing frequency of 'All Pegzilarginase Treated'; ties are sorted alphabetically within 'All Pegzilarginase Treated' column.
A Common TEAE is defined as a TEAE occurring in >=15% in the all peg treated group.

Adverse event of special interest

Three categories of TEAEs were considered as AESIs: hyperammonaemia, as this is known to be associated with ARG1-D, hypersensitivity, as this is a known risk for biologically manufactured therapeutic proteins and PEGylated substances and injection site reactions after SC injections.

Hypersensitivity (after IV)

In the DB phase of Study 300A, two subjects were reported to have events of hypersensitivity of mild-moderate severity versus none in the placebo arm.

In Study 101A, 4 of 16 subjects (25) had TEAEs with a PT of Drug hypersensitivity. For 3 of these subjects, this was rated as serious, and these were assessed by the Investigator as probably related to pegzilarginase. All events resolved within minutes to 6 hours, and dosing was completed.

Premedication with diphenhydramine, prednisolone and oral acetaminophen, interruption of dosing, and resumption of dosing at a lower infusion rate were effective methods of prophylaxis in all these subjects.

No cases of hypersensitivity were reported in LTE Study 102A or the LTE period of Study 300A.

Injection site reaction (after SC dosing)

Across the 102A and 300A LTE studies in the 34 subjects who received SC dosing with pegzilarginase (total of 1938 SC doses), the incidence of Injection site reactions was low (3/34 subjects, 8.8%), and no subjects discontinued pegzilarginase due to these events.

Hyperammonaemia

Per protocol, a hyperammonaemic episode was defined as a confirmed ammonia level ≥100 µM and 1 or more symptoms related to hyperammonaemia requiring hospitalisation or ER management. In addition, some investigators reported hyperammonaemia episodes that did not formally meet the above criteria if they observed symptoms considered to be consistent with a hyperammonaemic episode. These were also coded as Hyperammonaemia PT.

In the DB period of Study 300A, 3 of 21 subjects (14.3%) in the pegzilarginase arm and 4 of 11 subjects (36.4%) in the placebo arm experienced hyperammonaemia TEAEs. All but 1 of the TEAEs in these subjects were SAEs. The events were considered unlikely related or not related to the study drug except for one event. For 3 pegzilarginase subjects and 1 placebo subject, study drug dosing was interrupted due to the event. All events resolved with standard medical care. In the LTE period of Study 300A, 5 of 31 subjects (16.1%) had Hyperammonaemia TEAE.

In Study 101A, 6 subjects (37.5%) had TEAEs with a PT of Hyperammonaemia, of which at least 1 episode in each of these subjects were considered an SAE. In addition, in two subjects the nitrogen scavenger treatment was discontinued by the Investigator prior to the onset of the events. In long-term extension Study 102A, 6 of 14 subjects (42.9%) had hyperammonaemia TEAEs, and in 5 of the subjects, at least 1 event was serious.

The hyperammonaemia episodes were managed with standard medical care and considered resolved in all cases.

The rates observed in the placebo arm in the double-blind period of Study 300A (36.4%) and in the All Pegzilarginase Treated population (31.3%) were consistent with literature reports of ARG1-D patients (e.g. in natural history study UDCU, where 44% of the 16 ARG1- patients displayed HA episodes in on average 7 years follow-up).

In the long-term extension studies, the incidence rates tended to decline at continued use of pegzilarginase, despite that the daily protein intake increased up to 15%.

Table 23 Subjects with hyperammonaemia adverse events by preferred term for each 24 weeks period (full analysis set)

Period	MedDRA (Preferred term (PT)	Pegzilarginase Study 300A (N=21) n %	Placebo Study 300A (N=11) n %	Placebo-Pegzilarginase Study 300A (N=11) n %	Study 101A (N=16) n %	Study 102A (N=14) n %	All Pegzilarginase (n=48) n %
Overall	Subjects with AEs	7 (33.3)	5 (45.5)	5 (45.5)	6 (37.5)	9 (64.3)	22 (45.8)

0 < Weeks <= 24	Ammonia increased	4 (19.0)	2 (18.2)	2 (18.2)		3 (21.4)	9 (18.8)
	Hyperammonaemia	2 (9.5)	3 (27.3)	3 (27.3)	6 (37.5)	5 (35.7)	12 (25.0)
	Hyperammonaemic encephalopathy	1 (4.8)	1 (9.1)				1 (2.1)
24 < Weeks <= 48	Hyperammonaemia	2 (10.0)		1 (9.1)	1 (6.7)	2 (14.3)	5 (8.3)
48 < Weeks <= 72	Ammonia increased	1 (5.0)		1 (9.1)		2 (14.3)	4 (6.7)
	Hyperammonaemia			1 (9.1)		1 (7.1)	2 (3.3)
72 < Weeks <= 96	Ammonia increased			1 (9.1)		1 (7.1)	2 (3.3)
	Hyperammonaemia					1 (7.1)	1 (1.7)
96 < Weeks <= 120	Hyperammonaemia					2 (14.3)	2 (3.4)

Abbreviations: AE = Adverse Event; MedDRA = Medical Dictionary for Regulatory Activities.
Placebo-Pegzilarginase column initiated treatment with pegzilarginase after week 24
Percentages in the first row are based on the total number of subjects in each treatment group. Other percentages are calculated based on the numbers of active subjects at the beginning of each specified time period.
In the All Pegzilarginase column the AEs are counted only once per subject, meaning that this is not the sum for all studies as subjects transferred from Study 101A to Study 102A.
As of the cutoff, some patients have been treated for up to 196 weeks however no AEs of Hyperammonaemia have been reported after the 96-120 week interval.

2.6.8.3. Serious adverse event/deaths/other significant events

The most commonly reported SAEs were related to hyperammonaemia (13 out of 48 subjects, 27%). This was common both in the pegzilarginase and the placebo group in the DB phase of Study 300A.

Other frequently reported SAE were Hypersensitivity reactions (3 cases in Study 101A), all rated as probably related to pegzilarginase by the Investigator, and GI disorders (5 subjects, mainly nausea and vomiting). See Table 24 below.

All SAEs resolved with standard medical care, and none led to study discontinuation.

Table 24 Serious adverse events by MeDRA system organ class and preferred term

	DB Period: 300A		**101A (N=16)**	**Long-Term Extensions**		**All Pegzilarginase Treated (N=48)**
	Pegzilarginase (N=21)	**Placebo (N=11)**		**Study 102A (N=14)**	**Study 300A LTE (N=31)**	
System Organ Class Preferred Term	**Subjects n (%)**	**Subjects n (%)**	**Subjects n (%)**	**Subjects n (%)**	**Subjects n (%)**	**Subjects n (%)**
Subjects with any Serious TEAE	4 (19.0)	4 (36.4)	8 (50.0)	8 (57.1)	6 (19.4)	19 (39.6)
Gastrointestinal disorders	1 (4.8)	0	1 (6.3)	2 (14.3)	1 (3.2)	5 (10.4)
Vomiting	1 (4.8)	0	0	1 (7.1)	1 (3.2)	3 (6.3)
Abdominal pain	0	0	0	1 (7.1)	0	1 (2.1)
Nausea	0	0	1 (6.3)	0	0	1 (2.1)
Hepatobiliary disorders	0	0	0	0	1 (3.2)	1 (2.1)
Cholecystitis acute	0	0	0	0	1 (3.2)	1 (2.1)
Immune system disorders	0	0	3 (18.8)	0	0	3 (6.3)

Hypersensitivity	0	0	3 (18.8)	0	0	3 (6.3)
Infections and infestations	0	0	1 (6.3)	4 (28.6)	0	4 (8.3)
Gastroenteritis	0	0	1 (6.3)	2 (14.3)	0	2 (4.2)
Respiratory syncytial virus infection	0	0	0	2 (14.3)	0	2 (4.2)
Respiratory tract infection	0	0	0	1 (7.1)	0	1 (2.1)
Investigations	0	0	0	2 (14.3)	0	2 (4.2)
Ammonia increased	0	0	0	1 (7.1)	0	1 (2.1)
Aspartate aminotransferase increased	0	0	0	1 (7.1)	0	1 (2.1)
Liver function test increased	0	0	0	1 (7.1)	0	1 (2.1)
Metabolism and nutrition disorders	2 (9.5)	3 (27.3)	6 (37.5)	5 (35.7)	5 (16.1)	13 (27.1)
Hyperammonaemia	2 (9.5)	3 (27.3)	6 (37.5)	5 (35.7)	5 (16.1)	13 (27.1)
Nervous system disorders	1 (4.8)	1 (9.1)	0	1 (7.1)	0	2 (4.2)
Hyperammonaemic encephalopathy	1 (4.8)	1 (9.1)	0	0	0	1 (2.1)
Post-traumatic headache	0	0	0	1 (7.1)	0	1 (2.1)
Psychiatric disorders	0	0	1 (6.3)	0	0	1 (2.1)
Anxiety	0	0	1 (6.3)	0	0	1 (2.1)

Abbreviations: AE= adverse event; DB=double-blind; FAS=full analysis set; LTE=long-term extension; MedDRA=Medical Dictionary for Regulatory Activities; PT= preferred term; SOC= system organ class; TEAE = Treatment Emergent Adverse Event.

Notes: Percentages are based on the total number of subjects in the FAS. TEAEs are coded using the MedDRA SOC and PTs version 24.0. SOCs are sorted alphabetically. PTs are sorted by decreasing frequency of 'All Pegzilarginase Treated' column within each SOC; ties are sorted alphabetically within 'All Pegzilarginase Treated' column.

No deaths were reported in the clinical studies.

2.6.8.4. Laboratory findings

Haematology

No relevant trends were observed for haematology parameters like erythrocyte counts, hemoglobulin, and haematocrit.

Neither there were trends observed for platelet counts, mean platelet volume, prothrombin time, and activated partial thromboplastin time.

No trends were observed for immunology parameters, including leukocytes (neutrophils, basophils, eosinophils), lymphocytes, and monocytes.

Clinical chemistry

No trends were observed for albumin, calcium, glucose lactate dehydrogenase, phosphate, protein or prealbumin, or chloride, potassium, and creatinine.

Liver function tests (LFT)

In each study, mean values for LFTs, including ALT, AST, alkaline phosphatase and bilirubin, fluctuated over time but did not exhibit trends that were of clinical significance. Across studies, the number of subjects with clinically significant abnormalities remained stable over time. The majority of subjects who had LFT abnormalities had a history of liver dysfunction or elevated LFTs at baseline.

In the DB period in Study 300A, the incidence rates of increments of AST, ALT, bilirubin and normalised ammonia > ULN were similar among pegzilarginase and placebo arms (see Table 25 below).

Regarding the analyses of potential signals of liver injury, two subjects in the pegzilarginase arm met the criteria of ALT or AST >3× ULN, total bilirubin >2× ULN, although no ALP >2× ULN (in the DB and LTE period of Study 300A). In addition, both subjects had a medical history of AST/ALT or bilirubin elevations.

Table 25 Summary of abnormal liver function test criteria full analysis set; Study 300A DB phase

Category	Pegzilarginase n (%) (N=37)	Placebo n (%) (N=11)
AST > 3 x ULN	5 (13.5)	2 (18.2)
AST > 5 x ULN	4 (10.8)	2 (18.2)
AST > 10 x ULN	3 (8.1)	1 (9.1)
AST > 20 x ULN	1 (2.7)	1 (9.1)
Total Bilirubin > 2 x ULN	1 (2.7)	0

Ammonia

As discussed in the section of adverse event of special interest above, hyperammonaemia frequently occurred, although at the same rate as in the placebo group.

As shown in Table 26 below, there were no increments in the mean and median ammonia levels over time for the pegzilarginase treatment group. However, an increment was shown for the mean ammonia level in the placebo group at the end of the DB study phase (Week 24), but this may be related to outliers (two cases of ammonia increment of > 250 uM, Table 27). At longer-term treatment and after switching from placebo, the upper ammonia values were lowered, indicating fewer HA episodes.

Table 26 Normalised ammonia levels Study 300A

	Baseline Mean (SD); median (range)	Week 24 Mean (SD); median (range)	Week 48 Mean (SD); median (range)
Pegzilarginase	n=21 28.7 (19.9); 24.0 (6.0, 86.0)	n = 18 32.2 (16.8); 29.5 (11.0, 74.0)	n = 11 26.4 (8.7) 25.0 (18.0 47.0)
Placebo (week 48 pegzilarginase)	n = 11 28.4 (17.7) 25.0 (7.0, 56.0)	n = 6 79.5 (165.0) 21.0 (9.0, 544.0)	n = 6 40.2 (19.5) 37.0 (17.0, 73.0)

Table 27 Summary of ammonia values (full analysis set)

	Double-Blind Period: 300A		
Parameter(Unit)	Pegzilarginase n(%) N=21	Placebo n(%) N=11	Total Pegzilarginase + Placebo n(%) N=32
Ammonia (normalized to µM)			
Normal	8 (38.1)	4 (36.4)	12 (37.5)
ULN<xxx<=100 µM	10 (47.6)	3 (27.3)	13 (40.6)
>100 µM to <250 µM	3 (14.3)	2 (18.2)	5 (15.6)
>=250 µM to <500 µM	0	1 (9.1)	1 (3.1)
>=500 µM	0	1 (9.1)	1 (3.1)

Note: The categories are based upon the maximum Post Baseline value and include results from both scheduled and unscheduled visits.

Biomarker arginine

Based on the modelling and simulation, concentrations of arginine are expected to remain in the 40 to 115 µM range for 37% to 100% of the dosing interval for the majority of subjects with arginine concentrations of 100 to 200 µM 168-hour post-dose (i.e. just before the next weekly dose). See PK-PD section.

In Study 300A, 3 subjects had plasma arginine values <40 µM at 3-4 consecutive weekly measurements during IV dosing due to a lag between the availability of the arginine results for dose adjustments and the weekly visits.

2.6.8.5. In vitro biomarker test for patient selection for safety

Not applicable

2.6.8.6. Safety in special populations

The majority of the total pooled study population consisted of children and adolescents. Analyses of AEs were done for age groups of 2-5 y (n=8, 16.7%), 6-11 y (n= 16, 33.3%), 12-17 y (n= 16, 33.3%), and >=18 years (n=8, 16.7%). Overall, the number of AEs events was similar across the age groups (Table 28). HA and headache tended to be more common in adolescents and adults, but it is recognised that the subsets are very small and may not allow such analyses.

Table 28 Common TEAEs (occurring in ≥15% of subjects) by preferred term - by age group (full analysis set)

	Age Group 2-5 (N=8)		Age Group 6-11 (N=16)		Age Group 12-17 (N=16)		Age Group >=18 (N=8)		All Pegzilarginase Treated (N=48)	
Preferred Term	Subjects n (%)	Events	Subjects n (%)	Events	Subjects n (%)	Events	Subjects n (%)	Events	Subjects n (%)	Events
Any Common TEAEs	7 (87.5)	52	12 (75.0)	94	11 (68.8)	120	8 (100.0)	81	38 (79.2)	347
Vomiting	4 (50.0)	7	7 (43.8)	18	6 (37.5)	21	6 (75.0)	18	23 (47.9)	64
Cough	4 (50.0)	11	4 (25.0)	9	3 (18.8)	4	4 (50.0)	11	15 (31.3)	35
Headache	1 (12.5)	3	4 (25.0)	8	4 (25.0)	19	5 (62.5)	6	14 (29.2)	36
Hyperammonaemia	1 (12.5)	4	3 (18.8)	27	7 (43.8)	18	3 (37.5)	10	14 (29.2)	59
Ammonia increased	3 (37.5)	5	4 (25.0)	5	4 (25.0)	5	2 (25.0)	6	13 (27.1)	21
Nausea	1 (12.5)	4	1 (6.3)	2	5 (31.3)	13	5 (62.5)	16	12 (25.0)	35
Aspartate aminotransferase increased	2 (25.0)	5	4 (25.0)	5	3 (18.8)	8	2 (25.0)	2	11 (22.9)	20
Constipation	2 (25.0)	9	2 (12.5)	2	2 (12.5)	2	4 (50.0)	4	10 (20.8)	17
Alanine aminotransferase increased	1 (12.5)	1	3 (18.8)	4	3 (18.8)	12	2 (25.0)	2	9 (18.8)	19
Pyrexia	2 (25.0)	2	2 (12.5)	2	4 (25.0)	6	1 (12.5)	2	9 (18.8)	12
Upper respiratory tract infection	0	0	3 (18.8)	10	5 (31.3)	6	1 (12.5)	1	9 (18.8)	17
Abdominal pain	1 (12.5)	1	2 (12.5)	2	2 (12.5)	6	3 (37.5)	3	8 (16.7)	12

Abbreviations: TEAE = Treatment Emergent Adverse Event.
Note: Column denominators are used to calculate percentage.
AEs are coded using Medical Dictionary for Regulatory Activities (MedDRA) version 24.0.
PTs are sorted by decreasing frequency of 'All Pegzilarginase Treated'; ties are sorted alphabetically within 'All Pegzilarginase Treated' column.
A Common TEAE is defined as a TEAE occurring in >=15% in the all peg treated group.

No data are available from elderly patients. ARG1-D is associated with a reduced life expectancy.

The study population was equally distributed regarding gender, reflecting the general target population. There were no differences in nature and number of AES among male and female patients.

2.6.8.7. Immunological events

Routine monitoring of ADAs was implemented in all clinical studies. In addition, ADA assessments were obtained on demand during hypersensitivity reactions or if clinically indicated as determined by the Investigator.

Across studies, the ADA incidence was 25.0%, and the prevalence was 37.5%. In general, ADAs were transient and resolved with continued pegzilarginase treatment. ADAs developed early following the first IV administration for most subjects and resolved from the third dose onward. No subject has developed ADAs after switching from IV to SC administration thus far.

Of the 12 subjects who were ADA positive, 5 experienced hypersensitivity events. There was no clear relationship between ADA formation and injection site reactions, but only three cases were reported. No ADAs were found during SC administration.

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

No drug-drug interaction studies have been performed.

SmPC section 4.5 mentions that Loargys will interfere with routine arginine laboratory analysis, resulting in erroneous low measurements due to post-collection degradation of arginine.

2.6.8.9. Discontinuation due to adverse events

None of the patients who withdrew from the study were reported to be related to the study drug.

2.6.8.10. Post marketing experience

Not applicable.

2.6.9. Discussion on clinical safety

As might be expected for an ultra-rare disease, both the pooled dataset and the randomised dataset from Study 300A are very small, and therefore the frequencies of the adverse events and adverse drug reactions cannot be reliably assessed. Particularly the placebo group was small (n=11), and conclusions regarding safety signals of pegzilarginase versus placebo should be taken with care.

Pegzilarginase was generally tolerated well, although some patients (n=6, 12.5%) encountered infusion reactions after IV dosing of moderate severity. Infusion reactions were only reported in the first weeks of the studies. As none of these patients dropped out, it might be concluded that tolerance occurred. All the infusion reactions could be adequately treated with antihistamine agents (also prophylactic) and steroids. This has been adequately reflected in the SmPC.

Overall, infusion and injection site reactions may be considered the only adverse drug reactions observed thus far in this limited dataset. Most of the common AES, such as hyperammonaemia (HA), vomiting, abdominal pain, and liver function test elevations, which could be serious, could be considered disease-related. These events were also common in the placebo arm, and the patients had a history of LFT elevation and HA episodes before entering the study. There was no clear dose-response relationship for these AEs.

Infections were reported more frequently for pegzilarginase than placebo, but these mainly consisted of mild upper respiratory tract infections and were not considered treatment-related. As established by routine laboratory monitoring, pegzilarginase has no immunosuppressive properties, and the increased incidence may be a chance finding.

There were no consistent patterns of different safety profiles over the age groups.

Laboratory findings

The frequency of HA episodes tended to decrease in the LTE studies, although restriction of protein intake was relaxed in this phase of the study. It also declined after switching from placebo to active treatment. Although pegzilarginase does not directly target ammonia, it might indirectly impact ammonia formation due to the restoration of the urea cycle by the enzyme replacement of arginase-1. Although the decreasing trend is considered promising, the data are too sparse to draw final pegzilarginase treatment on hyperammonaemia.

As has been addressed in the SmPC, the use of nitrogen scavengers and the protein-restricted diet should be continued in ARG1-D patients at the use of pegzilarginase, which is supported.

There were no relevant trends observed for the haematology and clinical chemistry parameters.

Immunogenicity

As discussed in detail in the PK-PD section of this report, ADA's influenced PK and attenuated the PD effects on arginine, although this was transient. The development of an assay for detecting neutralising antibodies failed, but as arginine was measured weekly as a PD outcome, the neutralising effect of ADAs could be indirectly assessed.

Although it is known from the literature that small proteins (as in pegzilarginase) may be less immunogenic than large proteins like monoclonal antibodies, it may be unexpected that the detected ADA's circulated only for such a short period. No explanation could be found, but this issue is not pursued further as it is of limited clinical relevance.

Drug-induced hypoargininaemia

In the non-clinical studies in WT animals, significant drug-induced hypoargininaemia occurred. Anorexia and growth delay in juvenile animals, reduced reticulocytes, impaired fertility in both male and female animals, and learning and memory deficits in tests. The applicant expects that the effect of drug-induced hypoargininaemia in the non-clinical studies is not representative of the ARG1-D patients treated with pegzilarginase. Nevertheless, there were cases of prolonged hypoargininaemia in the pivotal trial. In three patients from Study 300A, arginine was persistently below the lowest limit of normality of <40 µM for 3-4 consecutive weeks, which was attributed to a lag time between the availability of the arginine results for dose tuning. It is not excluded that this may occur more often in clinical practice than in a more strictly regulated trial setting. Although no specific adverse events were noted in these three subjects, it is not excluded that this may develop at more prolonged hypoargininaemia. This was adequately addressed in the RMP.

SC dosing

Based on the data, SC dosing is better tolerated than IV dosing, with a reduced risk of hypersensitivity reactions and hypoargininaemia. This has been adequately reflected in the SmPC.

Long-term safety

The long-term safety data were limited, and rare events may have been unnoticed.

As pegzilarginase does not completely resemble the endogenous enzyme, off-target effects are not fully excluded. The applicant considers long-term safety as missing in the Safety Specifications in the RMP, which is agreed upon.

From the safety database all the adverse reactions reported in clinical trials have been included in the Summary of Product Characteristics.

Assessment of paediatric data on clinical safety

The main part of the study populations consisted of paediatric patients. The frequencies of AES were largely similar across age groups, but the subsets were too small to draw definitive conclusions. See under section special populations above for details.

Additional safety data needed in the context of a MA under exceptional circumstances

Safety exposure

The total safety database consisted of 48 subjects, and only 11 were assigned to placebo in the 24 weeks double-blinded phase of the main study. Such low numbers do not allow that frequencies of the adverse events and adverse drug reactions are reliably assessed. Rare or even common adverse events could have been unnoticed.

Safety: length of follow-up

The data are insufficient to establish long-term safety, particularly regarding the development of ADA's and related loss of efficacy or infusion/injection reactions.

The use of pegzilarginase resulted in -temporary- episodes of hyperargininaemia, particularly after IV dosing. Their clinical relevance is unclear but cannot be excluded based on the findings of the non-clinical studies, where drug-induced hypoargininaemia resulted in memory and learning deficits, and reduced male and female fertility.

2.6.10. Conclusions on the clinical safety

The available safety data available are sufficient to support a marketing authorisation. However, as the data are insufficient in order to establish long-term safety, the CHMP considers the following measures necessary to address the missing safety data in the context of a MA under exceptional circumstances (SOB):

- to fulfil the information gap on long-term safety, data will be provided from the ongoing LTE studies.

- a PASS within the existing ongoing E-IMD registry will be performed to obtain further information on Safety.

- in order to ensure adequate monitoring of safety and efficacy of pegzilarginase in the treatment of arginase 1 deficiency (ARG1-D) in adults, adolescents and children, the MAH shall provide yearly updates on any new information concerning the safety and efficacy of pegzilarginase.

2.7. Risk Management Plan

2.7.1. Safety concerns

Summary of safety concerns	
Important identified risks	None
Important potential risks	Severe hypersensitivity reactions Prolonged hypoargininaemia and its clinical sequela Medication errors during administration by a non-healthcare professional
Missing information	Safety in pregnancy and breastfeeding Long term safety

2.7.2. Pharmacovigilance plan

Study **Status**	**Summary of objectives**	**Safety concerns addressed**	**Milestones**	**Due dates**
Category 1 - Imposed mandatory additional pharmacovigilance activities which are conditions of the marketing authorisation				
None				

Study Status	Summary of objectives	Safety concerns addressed	Milestones	Due dates
Category 2 – Imposed mandatory additional pharmacovigilance activities which are Specific Obligations in the context of a conditional marketing authorisation or a marketing authorisation under exceptional circumstances				
A European, non-interventional, multicentre post authorisation safety study to evaluate the long-term safety of Loargys treatment in arginase 1 deficiency patients in standard clinical care (IMM-PEG-002) Planned	Evaluate the safety of pegzilarginase in the post-marketing setting	- Severe hypersensitivity reactions - Prolonged hypoargininaemia and its clinical sequelae - Medication errors during administration by a non-healthcare professional - Long-term safety - Safety in pregnancy and lactation	Protocol submission Interim reports	Within 3 months of EC decision Annually (with annual re-assessment)
Open-label extension study (Study CAEB1102-102A) Ongoing	Evaluate the efficacy and safety of pegzilarginase and characterize the PK and PD of pegzilarginase in patients ≥2 years for up to 4 years	- Severe Hypersensitivity reactions - Prolonged hypoargininaemia and its clinical sequelae - Long term safety	Final Report	31Mar2024
Category 3 - Required additional pharmacovigilance activities				
None None				

2.7.3. Risk minimisation measures

Safety concern	Risk minimisation measures
Important Potential Risks	
Severe hypersensitivity reactions	**Routine risk minimisation measures:** SmPC section 4.3, 4.4 and 4.8 PL section 2 and 4 Restricted medical prescription **Additional risk minimisation measures:** Educational material
Prolonged hypoargininaemia	**Routine risk minimisation measures:** SmPC section 4.2 and 4.4 PL section 3

Safety concern	Risk minimisation measures
and its clinical sequelae	**Restricted medical prescription** **Additional risk minimisation measures:** None
Medication errors during administration by a non-healthcare professional	**Routine risk minimisation measures:** SmPC section 4.2 PL section 3 and 7 **Restricted medical prescription** **Additional risk minimisation measures:** Educational material
Missing Information	
Safety in pregnancy and lactation	**Routine risk minimisation measures:** SmPC section 4.6 PL section 2 Restricted medical prescription **Additional risk minimisation measures:** None
Long term safety	**Routine risk minimisation measures:** **Restricted medical prescription** **Additional risk minimisation measures:** None

2.7.4. Conclusion

The CHMP considers that the risk management plan version 1.0 is acceptable.

2.8. Pharmacovigilance

2.8.1. Pharmacovigilance system

The CHMP considered that the pharmacovigilance system summary submitted by the applicant fulfils the requirements of Article 8(3) of Directive 2001/83/EC.

2.8.2. Periodic Safety Update Reports submission requirements

The requirements for submission of periodic safety update reports for this medicinal product are set out in the Annex II, Section C of the CHMP Opinion. The applicant did not request alignment of the PSUR cycle with the international birth date (IBD). The new EURD list entry will therefore use the EBD to determine the forthcoming Data Lock Points.

2.9. Product information

2.9.1. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use.*

2.9.2. Labelling exemptions

A request of translation exemption of the labelling as per Art.63.1 of Directive 2001/83/EC has been submitted by the applicant and has been found partially acceptable by the QRD Group for the following reasons:

All Member States (MS) agreed on an outer carton and vial label in English only.

A second request was to have only English, French, German and Spanish language printed package leaflet for all MS. A few MS requested a package leaflet to be printed and submitted along with the package in their national language. The remaining MS agreed to have a package leaflet in EN/FR/DE/ES only, with a suggestion to have a QR code in the printed PL that links the package leaflet in the local language.

The labelling subject to translation exemption as per the QRD Group decision above will however be translated in all languages in the Annexes published with the EPAR on EMA website, but the printed materials will only be translated in the language(s) as agreed by the QRD Group.

2.9.3. Quick Response (QR) code

A request to include a QR code in the package leaflet for the purpose of accessing a dedicated website has been submitted by the applicant and has been found acceptable.

The following elements have been agreed to be provided through a QR code: package leaflet and educational material.

2.9.4. Additional monitoring

Pursuant to Article 23(1) of Regulation No (EU) 726/2004, Loargys (pegzilarginase) 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
- it has a PASS imposed either at the time of authorisation [REG Art 9(4)(cb), Art 10a(1)(a), DIR Art 21a(b), Art 22a(1)(a)];
- it is approved under exceptional circumstances [REG Art 14(8), DIR Art (22)]

3. Benefit-Risk Balance

3.1. Therapeutic Context

3.1.1. Disease or condition

The approved indication reads: *"Loargys is indicated for the treatment of arginase 1 deficiency (ARG1D), also known as hyperargininemia, in adults, adolescents and children aged 2 years and older."*

ARG1-D is an ultra-rare inborn urea cycle disorder (UDC), a severe and chronic progressive metabolic disorder of the nitrogen pathway. The final enzyme reaction within the urea cycle is by ARG1

(arginase-1), which hydrolyses arginine into ornithine and urea. The kidneys excrete urea, whereas ornithine is returned to the mitochondria to continue the cycle. Inborn deficiency of this enzyme causes the accumulation of arginine and its toxic derivates (guanidino compounds and nitrous oxide), which are believed to be the key contributors to neurotoxic disease manifestations related to demyelination. Clinical manifestations typically start from about 2-4 years of age and include progressive neurological impairment with limb spasticity, which is considered the hallmark of the disease. In addition, cognitive developmental retardation and growth delay occurs. While acute and fulminant hyperammonaemia episodes are the most prominent hallmark feature in the other urea cycle disorders at neonatal age, these are generally less severe in ARG1-D. Hepatic impairment (leading to increased transaminases), vomiting and anorexia, and seizures are common, though not in every patient. Life expectancy is shortened.

The diagnosis is based on elevated concentrations of plasma arginine, deficient arginase 1 activity in red blood cells and molecular genetic testing. More than 60 ARG1 gene mutations have been described in the literature.

ARG1-D is one of the least common urea cycle disorders (UCD), accounting for approximately 3.5% of all UCD cases. It is an ultra-rare disease with an estimated incidence of approximately 1:300,000–1:2,000,000 live births globally (Sin 2015). The incidence rates seem similar over continents. It is estimated that 4-5 newborns in the European Union are born with ARG1-D (Burrage, 2015). The applicant is aware of 70-80 patients diagnosed with ARG1-D in the EU.

Pegzilarginase is an enzyme replacement therapy (ERT), intended to substitute for the deficient human arginase 1 enzyme activity in patients with ARG1-D. The active substance is a complex of a pegylated recombinant human Arginase 1, where the manganese enzyme co-factor has been replaced with cobalt, to enhance stability. The product is pegylated to extend the half-life. Loargys should be given in conjunction with individualised disease management, such as dietary protein restriction with essential amino acid supplements and pharmacological treatment, including nitrogen scavengers. The initial starting dose is 0.1 mg/kg weekly, either by IV infusion or subcutaneous injection. Guided by the response of the individual plasma arginine levels, the dose can be adjusted to 0.05-0.2 mg/kg in dose steps of 0.05 mg/kg.

3.1.2. Available therapies and unmet medical need

Current management approaches for ARG1-D mainly consist of dietary protein restriction, with essential amino acid (EAA) supplementation. International guidelines for ARG1-D focus on the reduction of plasma arginine to levels of <200 µM (Häberle 2019) and ideally to within the normal range (less than 115 µM) (Lüneburg 2011) as the ultimate treatment goal. Nitrogen scavengers are applied to control excessive ammonia levels if present. Liver transplantation has been reported to lead to the normalisation of arginine and ammonia levels and amelioration of cognitive and motor function delay in several cases.

However, in the majority of patients, the stringent protein-restricted diet does not lead to a sufficient reduction of arginine below the target levels. The genesis of arginine in the human body depends on whole-body protein turnover, and the level of arginine is only partially dependent on dietary intake of arginine. It has been estimated that about 20-25% of the natural human arginine is derived from the diet (Zhou M, Martindale RG. J Nutr. 2007;137(6 Suppl 2):1687S-1692S). Moreover, the diet is poorly palatable and difficult to manage.

Liver transplantation is available to only a small fraction of patients and is associated with significant risks.

Pegzilarginase is an enzyme replacement therapy that reduces arginine to normal limits in ARG-1 deficient patients, potentially reducing the risk of development or progression of neuromotor and cognitive impairment.

3.1.3. Main clinical studies

The first part of multi-centre Study CAEB1102-300A (Study 300A) consisted of a 24-weeks randomised, double-blinded, placebo-controlled study phase. Patients were eligible with an established diagnosis of arginase 1 deficiency (genotype or RBC), and arginine plasma levels ≥250 uM. Patients were stratified based on a history of recent previous hyperammonaemia episodes. Subjects were randomised 2:1 to receive pegzilarginase or placebo intravenously once weekly at an initial dose of 0.1 mg/kg. Subjects were individually titrated within a 0.05 – 0.2 mg/kg range, guided by their plasma arginine levels. All subjects were to continue on any previously prescribed dietary regimen and nitrogen scavengers throughout the study period. In total, 32 subjects were included (21 assigned to pegzilarginase, 11 to placebo). The median age was 10.5 years (range 2, 29). The primary endpoint assessed the reduction from baseline in plasma arginine in subjects treated with pegzilarginase compared to placebo at Week 24. The key secondary endpoints assessing functional mobility were Gross Motor Function Measure Part E (GMFM-E), which measures the subjects' ability to walk, run, and jump, and the 2-minute walking distance test (2MWT). The randomised part of the study is completed. All 31 patients who finished the 24 weeks randomised part were eligible for the long-term extension (LTE) study with pegzilarginase, which is ongoing. The first 8-weeks of the LTE remained blinded. After 8 weeks in the LTE, patients could be switched to SC dosing in the open-label extension study.

Study CAEB1102-101A (Study 101A) is a Phase 1 /2 open-label uncontrolled dose-finding study in 16 paediatric and adult patients with ARG1-D (median age 15, range 2-31y). In part A, single IV doses were individually titrated every two weeks till arginine target level of < 200 uM was achieved, followed by part B, where the optimised dose (0.03-0.2 mg/kg IV) was repeated for 7 weeks. From this study, 14 subjects entered the open-label extension study 102A, which is still ongoing and the results will be provided in the post-marketing setting. In the extension study, patients could be switched to an equivalent SC weekly dose.

3.2. Favourable effects

In all studies, pegzilarginase rapidly and sustainably reduced the arginine levels, generally well below the clinical target level of 200 µM, and the more critical upper normal level of 115 uM.

In Study 300A, the mean (SD) plasma arginine levels dropped from 354.0 (1.30) µM at baseline to 86.4 (1.6) µM at Week 24 in the pegzilarginase arm, whereas in the Placebo arm, the mean baseline arginine levels remained more or less stable (464.7 (1.21) µM at Baseline and 426.5 (1.31) at Week 24). Pegzilarginase induced a 76.7% (95% CI 67.1%, 83.5%) reduction in GLS mean ratio for change of plasma arginine at week 24 from baseline relative to placebo ($p<0.0001$). Thus, the primary objective was met.

For 90.5% of the subjects in the pegzilarginase group, the arginine levels at Week 24 were below the clinical target level of 200 µM and ≤115 µM. None of the subjects in the placebo group reached the

arginine target levels (nominal p<0.0001, Fisher exact test). Maintenance of the low arginine levels within the target range was observed in patients switching to SC dosing.

Alongside arginine reduction, the mean plasma levels of the neurotoxic metabolites of arginine, i.e. four guanidino compounds that could be analysed, were reduced by approximately 50-70% from baseline after 24 weeks of pegzilarginase treatment. In contrast, these remained unchanged or slightly increased in the placebo arm.

Within 24 weeks, a numerical trend of improvement was consistently shown in multiple motor function outcomes in favour of pegzilarginase. The key secondary endpoint, GMFM-E, overall improved from Baseline to Week 24 for the pegzilarginase treatment arm (4.2 points), whereas it declined in the placebo group (-0.4 points, LS mean difference 4.6 (95% CI -1.1, 10.2)). In addition, a numerically small improvement was observed for 2MWT (LS mean change from BL at Week 24: 7.4 versus 2.9 for placebo, difference 5.5 m (-15.6, 26.7). Several secondary and tertiary endpoints pointed in the same direction, including GMFM-D (measuring motor function of standing), Gillette Functional Assessment Questionnaire (a PRO), Vineland Adaptive Behaviour Scale, and paediatric QoL scales.

Responder analyses based on the minimal clinically important difference (MCID) of 2MWT, GMFM-D and GMFM-E, which were pre-specified based on the literature, 6 out of 17 patients of the pegzilarginase treated group who were eligible for MCID analyses, achieved MCID thresholds for at least two motor function scales after 24 weeks (either GMFM-D, -E or 2MWT), versus none for placebo (no statistics provided). The MCID response continued to improve in the long-term extension phase of Study 300A (12/17 patients who were initially assigned to pegzilarginase and 2/9 in patients switching from placebo).

Five patients from Study 300A with the most severe motor impairment at baseline (GMFCS level IV) were necessarily excluded from the MCID analyses, as no MCID value has been defined in the literature for this severity level. These most severely impaired patients also tended to improve; three of five subjects improved to level III at their last assessment. Level III means that they still need walking aids but without the need of continuous assistance of caretakers at mobility (level IV). For these 3 subjects, this was accompanied by an increase in 2MWT distance and GMFM-D score that exceeded the MCID thresholds.

In pre-planned subgroup analyses based on the severity of motor function at baseline, the treatment effects regarding the key-secondary endpoints (2MWT, GMFM- E) were larger for pegzilarginase in patients with moderate-severe impairment at Baseline (GMFCS Level ≥II), than in subjects classified as GMFCS Level I, who had mild deficits at Baseline (not formally tested).

In the long-term extension study 102A, the motor function scores continue to improve from baseline. At Week 48, the mean change from baseline was 44.9 m (95% CI 18.6, 71.3) for the 6MWT, 5.8 (95% CI 2.5, 9.1) points for GMFM-E, and 2.8 (1.2, 4.3) for GMFM-D. Plasma arginine levels also remained low and did not exceed the clinical target in the long term (48 weeks or longer).

The incidence of hyperammonaemia episodes declined at continued treatment with pegzilarginase in the long-term extension studies, even though an increment of the daily dietary intake of 15% was allowed.

3.3. Uncertainties and limitations about favourable effects

There is only one single randomised study. The sample size was small, and the benefits on motor function have not been formally confirmed at a statistically significant level. Furthermore, there was no

improvement in Global Impression Clinical Change scores (either by clinician or caretakers/patients) as compared to placebo. However, the randomised period of 24 weeks might have been too short to demonstrate a relevant improvement of longer follow-up data from the long-term extension studies showed an improvement in motor function or stabilisation in majority of ARG1-D patients, which would normally not be expected for this progressive degenerative disorder.

Although the guanidino compound levels significantly dropped from baseline after 24 weeks of pegzilarginase treatment, these were still above the levels reported for healthy people. Longer follow-up data showed that that GCs levels remained low as compared to baseline, with levels below or near normal for the different compounds.

The 6MWT endpoint of this study is not corrected for ageing/growth effects in children. Therefore, the age-adjusted GMFM scores may be considered more informative for long-term outcome.

No data is available in patients older than 32 years, although survival may be more prolonged in this disease. It is unclear whether the study outcomes can be extrapolated to older patients with advanced disease, as the neurological damage, such as spastic diplegia and cognitive disabilities, may not be reversible in these patients. This has been adequately reflected in the SmPC.

3.4. Unfavourable effects

Clinical safety data are available from 48 ARG1-D patients (age range 2-32y, median 11.5 y), who received 1270 IV doses and 1938 subcutaneous (SC) doses. The mean (SD) duration of exposure at data cut-off was 71.7 (55.03) weeks. Over 50% of the 48 subjects have been treated with pegzilarginase for >1 year as of the data cut-off dates for the studies (maximum of 3.8 years).

In the randomised, double-blind phase of Study 300A, AEs were reported in 85.7% of the pegzilarginase am (18/21), and 100% of the placebo group. Serious AEs were reported in 19% (4/21) in the pegzilarginase arm versus 36.4% (4/11) in the placebo arm. For pegzilarginase, the SAEs included 3 cases of hyperarginaemia and one case of vomiting, and for the placebo group, these were four cases of hyperammonaemia.

Withdrawals were not considered to be treatment-related. There were no fatalities during the studies.

Adverse drug reactions that were observed for pegzilarginase were hypersensitivity reactions (at IV infusion), injection site reactions at SC use and ADA formation. Six of 48 (12.5%) Loargys-treated subjects (pooled dataset) experienced 1-3 episodes of hypersensitivity reactions when administered intravenously. Commonly observed signs and symptoms included rash, facial swelling, flushing, shivering, cough, abdominal pain, and dyspnoea. Three cases were considered serious. All the hypersensitivity reactions could be handled with standard of care, and none led to treatment withdrawal, and treatment was restarted at a reduced infusion rate within hours. Prophylaxis with antihistaminic agents (IV or oral) and/or prednisone were given for the following doses.

Injection site reactions were reported in 8.8% (3/34) of patients after subcutaneous administration. However, none of these was considered serious.

Across studies, the ADA incidence was 25.0%, and the prevalence was 37.5%. In general, ADAs were transient and not detected at continuous treatment. ADAs developed early following the first IV administration for most subjects and resolved from the third dose onward. No subject developed ADAs after switching from IV to SC administration. ADA's, either against arginase or the PEG component of the drug, interfered with PK and efficacy (higher arginine levels). There was no clear relationship between ADA formation and adverse events like hypersensitivity.

Other adverse events that were commonly reported for pegzilarginase, such as hyperammonaemia, liver enzyme increments, and nausea/vomiting, were also frequently reported for placebo and are known symptoms of the disease.

Hyperammonaemia and liver enzyme increments were pre-defined as AEs of special interest. Overall, in the pooled dataset of all 48 subjects exposed to pegzilarginase, 15 patients experienced one or more episodes of hyperammonaemia (serious and non-serious). In the double-blind phase of Study 300A, the incidence of SAEs due to hyperammonaemia was 3/21 in the active treatment group vs 3/11 in the placebo group. All of these cases had a history of recurrent episodes of hyperammonaemia. A decreasing trend over time was observed in the LTE study.

Liver Function Test (LFT) increments were very common and balanced with the placebo group. In the routine monitoring, the most common abnormality was ALT >3× ULN, which occurred in approximately 50% of the subjects of Study 300A, equally distributed over the study arms. Bilirubin increments were incidental and of moderate severity. Most subjects with LFT abnormalities had a history of liver dysfunction or elevated LFTs at baseline.

In the double-blind period of Study 300A, 8 subjects in the pegzilarginase arm (38.1%) reported Infections and infestations compared to 1 subject (9.1%) in the placebo arm. These were mostly reported as viral infection of the upper respiratory tract, none were reported to be treatment-related, and all were reported in children and adolescents, and not in adults.

No seizures were reported in the double-blind study phase (about 30% in the randomised study 300A had a history of seizures at baseline). There were no noticeable effects of pegzilarginase on ECG, EEG or vital functions at routine monitoring.

The non-clinical studies do not indicate a vacuolisation risk of the PEG compound. Besides, vacuolisation is not expected based on the low dose and the low molar weight of the PEG component of this drug (below the critical level of 40 kDa).

3.5. Uncertainties and limitations about unfavourable effects

Overall, the safety database is very limited. This hampers the assessments of the frequencies of the AEs, and to establish adverse drug reactions versus the placebo.

In the non-clinical studies for this product using wild-type animals, the use of pegzilarginase lead to significant hypoargininaemia. These models are thus not considered representative for the patients. In the hypoarginaemic animals, learning and memory deficits, anorexia and weight loss, lower erythrocyte and monocytes, female and male infertility, and reduced spermatogenesis were observed.

The IV dosing regimen in ARG1-D patients with hyperarginaemia leads to steeper plasma arginine reduction, regularly below the lowest limit of the normal reference value of 40 uM. In Study 300 A, three subjects out of 21 had plasma arginine values <40 µM, at 3-4 consecutive weekly measurements during IV dosing. The clinical consequences of the transient hypoargininaemia at IV dosing are unclear. This has been adequately addressed in the RMP, Safety Specifications. Moreover, SC administration significantly lowered the duration and incidence of hypoargininaemia episodes.

No clinical data are available in pregnancy, and this has also been addressed in the RMP.

3.6. Effects Table

Table 29 Effects table for Loargys, treatment of arginase 1 deficiency (ARG1-D)

Effect	Short Description	Unit	Treatment pegzilarginase	Control placebo	Uncertainties/ Strength of evidence	References
Favourable Effects						
pARG W24 (primary)	Percent reduction in GLS mean at week 24 compared to baseline	%	76.7 (95 % CI-146.7, 300.1)	0.0 (95% CI -234.4, 232.4)	SoE 76.6 (95% CI 67.1, 83.5) ratio for change (treatment/placebo), P<0.0001 SoE Responder rates (<115uM) 90.5% and 0 for pegzilarginase and placebo, resp. at W24 (95% CI difference 58.7, 98.8, nominal p-value <0.001)	300A
GMFM-E (walking, running, jumping)	LS Mean change from BL W24[1]		4.2 (95% CI NR)	-0.4 (95% CI NR)	UN: LS Diff (95% CI) 4.6 (-1.1, 10.2) p= 0.1087 UN: Similar trend for GMFM-D domain on standing: 2.3 (-0.4, 4.9)# SoE : change from BL to-W48; 5.8 (95% CI 2.5, 9.1) from long-term uncontrolled study SoE change from BL to-W48; GMFM-D (standing) 2.8 (1.2, 4.3)	300A 102A
2MWT	LS Mean change from BL[1]	m	7.4 (95% CI NR)	1.9 (95% CI NR)	UN LS Diff (95% CI) 5.5 (-15.6, 26.7) p = 0.5961 SoE : change from BL to-W48; 44.9 (95% CI 18.6, 71.3) from uncontrolled study	300A 102A
Unfavourable Effects						

Hyper sensitivity	incidence	%	2 (9.5)	0 (0)	Rated as non-serious 4 hypersensitivity cases (3 SAEs) None of cases led to treatment withdrawal, all were transient (1-3 dosing occasions), and responded to SoC.	300A 102A
Hyperammonia (HA) episodes	incidence	%	3 (14)	3 (27)	SoE; all patients had a history of HA episodes at baseline Incidence of HA episodes further declined in the LTE studies	300A

Abbreviations: 2MWT= two minutes walking test, 6 MWT= 6 minutes walking test, BL = baseline, GMFM-D = Gross Motor Function Measure-Item D (standing); GMFM-E = Gross Motor Function Measure-Item E (walking, running and jumping); HA = hyperammonia;NR = not reported;; pARG= plasma arginine level, SoC = SoC with antihistamine agents or prednisone, SoE: strength of evidence, W =Week
Notes: # excluding an outlier in the placebo-group due to an erroneous scoring of 0 for a missing measurement at BL,

3.7. Benefit-risk assessment and discussion

3.7.1. Importance of favourable and unfavourable effects

Pegzilarginase, as an enzyme replacement, has been shown to reduce hyperarginaemia effectively, in patients with an arginase-1 deficiency, to the extent of normal arginine reference values in a high percentage of patients. The PD effect of the reduction of arginine was durable.

The standard of care of a protein-restricted diet fails to reduce the arginine levels below the clinical target as defined in an international treatment guideline (<200 uM), in the majority of patients because arginine is formed endogenously from other proteins and dietary intake of arginine only contributes for a small part to the total body flux of arginine. The proposed posology adequately reduces arginine levels below the treatment target level.

Given the strong pharmacological rationale of enzyme replacement therapy and the ultra-rarity of the disease, it was accepted that a single randomised controlled trial was performed. A similar reduction in arginine levels by pegzilarginase as in the confirmatory trial has also been established in the open-label Phase 1 / 2 study.

The surrogacy of the primary pharmacodynamic endpoint of plasma arginine levels was discussed in a comprehensive review of the literature. Hyperarginaemia has been shown to lead to demyelination of the pyramidal tract in animal models of the disease and in patients. The neurotoxic effects of hyperarginaemia are also mediated by the accumulation of its toxic metabolites, the guanidino compounds, and an excess of nitrous oxide formation. There is a direct correlation between arginine and GC levels in plasma and CSF.

The analyses from a prospective natural history study from 6 patients with follow-up of 6-8 years demonstrate that cumulative higher arginine levels were correlated with symptoms severity and poorer prognosis regarding the developmental cognitive delay, behaviour problems and spasmic paraplegia. Furthermore, after orthotopic liver transplantation, an amelioration of the progression of neurological and cognitive impairment was reported in patients who normalised arginine and ammonia levels. Other studies did not directly correlate arginine levels and disease severity, but these were retrospective or cross-sectional (Huemer, 2016). Animal models of ARG1-D support that normalisation of arginine by other methods (such as ARG1 gene replacement therapy), leads to rescue of neural demyelination and prolonged survival. All these data from the literature support the rationale of targeting hyperarginaemia as a treatment goal.

Hyperammonaemia is a common feature of this disease, which is also associated with overlapping symptoms such as seizures, aberrant behaviour and cognitive delay - albeit not spastic paraplegia, which is specifically related to hyperarginaemia. Due to its mode of action, pegzilarginase does not directly target ammonia levels. Nevertheless, the incidence of hyperammonaemia episodes declined at continued treatment with pegzilarginase in the LTE studies, even at increased dietary protein intake. This might be related to restoration of the whole urea cycle by the enzyme replacement therapy with pegzilarginase. However, the applicant considers that the data are too sparse to allow recommendations in the SmPC to relax the dietary restrictions and the use of nitrogen scavengers, which is supported.

Pegzilarginase also significantly reduced the levels of guanidino metabolites of arginine, which several experts consider important drivers of neurotoxicity in ARG1-D patients, perhaps more important than arginine itself. However, the clinical relevance remained unclear, as although reduced, the plasma levels of some GC-compounds were still above the normal upper level.

Although arginine as the primary endpoint for the main study was accepted by the SAWP/CHMP in their advice, it was emphasised that this should be supported by clinical outcomes (preferably as co-primary). However, the key secondary outcomes on motor function failed to meet their endpoints. Although a clear trend in the direction of a positive effect of pegzilarginase compared to placebo was observed for the gross motor function measures (GMFM-D and E), the outcomes of the 2MWT response were less conclusive. Patients/caretakers did not report notable clinical changes for either pegzilarginase or placebo in GCI scores either.

The treatment effects concerning GMFM-D, -E (and 2MWT) were numerally larger in the patients with more severe gross motor function impairment at baseline, as was consistently shown in both studies (102A and 300A). The modest improvement in motor scores in patients with mild motor function impairment at baseline (50% of the study population) was attributed to a ceiling effect. This reasoning is accepted.

However, the data from this long-term study indicate that the 24-week placebo-controlled period of the main trial may have been too short to cover the optimal treatment effect on motor function outcomes. For ethical considerations, the CHMP/SAWP agreed with the 24-week placebo control. It is considered promising that at continued treatment in the LTE studies, the MCID responder rates gradually continued to improve, also in patients switching from placebo. Whereas without treatment, a gradual decline of motor function would be expected in this chronic, progressive metabolic disorder, which cannot be adequately controlled with a standard of care (diet and nitrogen scavengers) alone under the normal circumstances.

Also supportive is the fact that 3 out of the 5 most severe patients at baseline (GMFSM level IV), where no MCID has been defined in the literature as no improvement may be expected, improved at long-term follow up in GMFM-D (standing) and 2MWT, and they did not require constant assistance of the caretaker anymore in terms of mobility.

It is also noted that several promising features were observed in the open-label long-term phase 2 study, showing an increasing improvement of gross motor function and the 6-MWT from baseline at continuous treatment for at least 48 weeks. While the improvement in 6MWT may also be due to aging of the paediatric patients at such a long-term follow-up, the GMFM score is corrected for age. Although obtained from an uncontrolled study, the data are considered supportive.

Patients with a relatively modest level of hypoarginaemia (<250 uM), were excluded from the studies. According to the wording of the indication, these patients are eligible for treatment. Case reports of patients who achieve arginine levels near the target level of 200 uM with a diet alone, described that they did not develop motor impairment. However, based on the literature, it is known that the toxic GC metabolites may still accumulate above normal levels, even though the arginine levels could be reasonably controlled in some patients near the clinical target levels. Although there is a rationale to target the arginine levels within the physiological boundaries as toxicity may still occur due to long-circulating GC, the clinical benefits of Loargys in patients with arginine levels below or near the clinical threshold of 200 µM remain somewhat unclear since these patients were excluded from the trial, and their symptoms may be modest. The decision for treatment should be made on an individual basis. This has been adequately addressed in the SmPC.

The study population mainly consisted of children and adolescents and only a few young adults, which is understood as younger patients may benefit most from early intervention in this progressive neurodegenerative disease. However, patients reaching middle age have been reported in the literature. It remains unclear to what extent the observed clinical treatment effects from adolescents and young adults can be extrapolated to older patients with long-existing neurological damage that might not be reversible. This has been adequately addressed in the SmPC and will be monitored post-marketing in the registry.

According to the posology, the dose should be individually titrated based on the patient's arginine plasma levels. A warning is included in the SmPC regarding the fact that Loargys will interfere with routine arginine laboratory analysis, resulting in erroneous low measurements due to post-collection degradation of arginine. As clearly mentioned in the SmPC, validated methods should be used to measure plasma arginine levels reliably. The applicant has committed that they will provide the specifically prepared CE marked tubes with an enzyme blocker, the same as applied in the studies, to the clinics after marketing authorisation approval.

In general, pegzilarginase was tolerated in the studies, and no patients dropped out due to adverse events. Infusion and injection site reactions have been reported, but these were transient and could be adequately handled with precautionary measures. These measures are adequately addressed in the SmPC.

Based on the non-clinical data and given the low molecular weight of the pegylation compound of the drug, the risk of potentially harmful accumulation of the pegylated compound seems to be low. The active compound of the drug largely resembles the endogenous enzyme, and no off-target PD effects are known. Currently, no important safety issues are identified with the limited data available. The main adverse drug reaction was infusion reactions at IV use. These did not lead to treatment withdrawal and could be adequately addressed with infusion rate reduction and antihistamine agents, which is reassuring.

ADA formation frequently occurred, although transient and not associated with serious consequences thus far. The long-term follow-up data do not indicate an increasing trend of ADA formation, but the data are too sparse to exclude this fully. A PASS will be conducted that will monitor ADA related adverse events, such as reduced drug activity and hypersensitivity reactions.

Data are lacking on whether the current upper pegzilarginase dose would compensate for dietary non-adherence. Also, ADA formation may require higher doses than tested so far. This will be addressed in the planned post-marketing registry (PAES).

Transient episodes of hypoargininaemia (<40 uM) were observed in the patients, particularly after IV dosing, but much less after SC administration. The clinical consequences of transient periods of hypoargininaemia are not clear. The non-clinical WT animal models showed significant toxicity due to pegzilarginase-induced hypoargininaemia, including memory and learning deficits and anaemia. Modelling and simulation exercises supported that starting with SC administration would reduce hyperarginaemia similarly to initial IV administration but with a considerably lower risk of hypoargininaemia episodes. SC dosing has been added in the SmPC, and also a recommendation have been added for at-home administration.

Although the available data thus far do not indicate specific important safety issues and are considered sufficient to support the granting of a marketing authorisation, it is noted that the safety database is very small, and frequencies of the adverse events and adverse drug reactions could not yet be reliably assessed. As a result, rare adverse events could have been unnoticed. Therefore, a PASS will be performed.

From a quality perspective, the applicant has agreed to post-approval commitments on drug product specifications and pegylation.

3.7.2. Balance of benefits and risks

Pegzilarginase significantly reduced hyperarginaemia in patients with arginase-1 deficiency to the level of normal ranges. This can normally not be achieved with the standard of care of a protein-restricted diet with nitrogen scavengers. No other pharmacological treatment options are available to reduce hyperarginaemia, and there is an unmet medical need. Clinical data indicating a relevant improvement of the gross motor function at long-term treatment, even in patients with severe motor impairment at baseline, support the surrogacy of the primary pharmacodynamic endpoint. Normally, a deterioration of motor function is expected in this progressive disorder.

Although the study data are too limited to reliably estimate the frequency of adverse events in this ultra-rare disease, the identified safety risks of hypersensitivity and injection site reactions are considered acceptable and manageable in clinical practice.

The applicant has made adequate commitments (SOBs) to obtain additional efficacy and safety data in the post-marketing setting, also making use of an existing European registry.

Altogether, the benefit-risk balance is considered positive.

3.7.3. Additional considerations on the benefit-risk balance

Marketing authorisation under exceptional circumstances

As comprehensive data on the product are not available, a marketing authorisation under exceptional circumstances was proposed by the CHMP during the assessment, after having consulted the applicant.

The CHMP considers that the applicant has sufficiently demonstrated that it is not possible to provide

comprehensive data on the efficacy and safety under normal conditions of use, because the applied for indication is encountered so rarely that the applicant cannot reasonably be expected to provide comprehensive evidence.

Assessment of the criteria of comprehensiveness

1. Quality of evidence (including feasibility considerations)

Despite the rarity of the disease, a randomised placebo-controlled trial was performed, which is supported. The main study was adequately designed and powered to demonstrate the *primary* objective, i.e. the potency of pegzilarginase to reduce hyperargininaemia as compared to the standard of care alone (add on to placebo).

Regarding the key secondary objectives: The main study might have been inadequately powered and is considered of too short duration given the slowly progressive nature of the disease to fully establish the clinical effects of the surrogate primary endpoint.

2. Efficacy: precision of effect size

The precision of the effect size of pegzilarginase on the mean reduction of arginine from baseline compared to placebo could be adequately estimated.

Although a tendency for improvement was consistently shown for gross motor function outcomes in favour of pegzilarginase, the effect sizes of the (key) secondary outcomes could not be precisely quantified, as they did not meet statistical significance. This study might have been inadequately powered or of too short in duration to establish the clinical effect size.

3. Efficacy: clinical meaningfulness of the endpoint

Treatment goals as defined in international treatment guidelines (arginine =<200 uM arginine OR normal reference value 40-115 uM) were achieved.

The surrogacy of plasma arginine levels for clinical benefit has not been formally confirmed in the main trial since the key-secondary clinical outcomes failed to meet their endpoints at Week 24. In the long-term extension study, there are tendencies for continued improvement in motor function, at the level of MCID.

Some experts consider that the GC compounds are largely responsible for the neurotoxic effects of the disease. The plasma levels of these toxic metabolites of arginine significantly reduced from baseline when using pegzilarginase, but their clinical relevance remained unclear as some of these compounds were still above normal reference values at long-term follow-up.

4. Efficacy: duration of efficacy

The disease is slowly progressive. The long-term follow-up study 102A in 13 patients who could be followed for 48 weeks showed durable decreased arginine levels and consistent improvement from baseline in gross motor function. Though promising, these numbers are considered too small to exclude the risk of antibody formation and, therefore, reduced efficacy. Neither was a contextualisation of these long-term outcomes provided by controls (e.g. 6MWT may be influenced by growth and development at longer-term follow-up in children).

The PK-PD modelling predicted that 3% of the patients might require a higher dose than the maximal dose of 0.2 mg/kg that was applied in the studies.

The adherence to the protein-restricted diet may decrease in daily practice, particularly once the arginine levels are stabilised. It is unclear whether the proposed dose range in the SmPC will be adequate to address this. Higher doses may also be required to overcome ADA effects.

5. Safety: exposure

The total safety database consisted of 48 subjects, and only 11 were assigned to placebo in the 24 weeks double-blinded phase of the main study. Such low numbers do not allow for the frequencies of the adverse events and adverse drug reactions to be reliably assessed. Rare or even adverse events could have been unnoticed.

6. Safety: length of follow-up

The non-clinical data do not indicate a considerable risk of vacuolation due to the PEG compound of pegzilarginase.

Nevertheless, the data are insufficient to establish long-term safety, particularly regarding the development of ADA's and related loss of efficacy or infusion/injection reactions.

The use of pegzilarginase resulted in -temporary- episodes of hypoargininaemia, particularly at IV use. Therefore, their clinical relevance is unclear but cannot be excluded based on NC studies, where drug-induced hypoargininaemia resulted in memory and learning deficits and reduced male and female fertility.

7. Target population vs study population

ARG1-D patients with mildly increased arginine levels were excluded from the main study, and neither were included in the Phase 1/2 studies.

Information is lacking in middle-aged and elderly adult patients. In addition, the reversibility of symptoms may be limited in adult patients with long-existing neurological damage.

The studies excluded patients younger than the age of 2 years. Therefore, dosing in younger patients (birth-2 years of age) will be addressed in an ongoing Safety and PK study in line with PIP requirements.

8. Pharmacological rationale

The product is an enzyme replacement therapy, thereby in principle, it may provide a strong pharmacological rationale.

Some experts consider that the neurotoxicity is mainly driven by the guanidino metabolites of arginine rather than arginine itself. GC plasma levels were also reduced from baseline at the use of pegzilarginase, but it is unknown what would be the relevant target value for these biomarkers.

Due to its mode of action, pegzilarginase has no influence on other aspects of the disease, such as LFT increments.

9. Natural history/ course of the disease

Sufficient data from the literature has been provided on the time of diagnosis and the natural disease course.

Although it would be challenging to obtain comprehensive data because of the rarity of the disease, additional efficacy and safety data will be gathered post-marketing. This will be obtained from a PAES and PASS in the context of an existing European registry (E-IMD) for metabolic disorders.

Therefore, recommending a marketing authorisation under exceptional circumstances is considered appropriate.

3.8. Conclusions

The overall benefit/risk balance of Loargys is positive, subject to the conditions stated in section 'Recommendations'.

4. Recommendations

Similarity with authorised orphan medicinal products

The CHMP by consensus is of the opinion that Loargys is not similar to Ravicti within the meaning of Article 3 of Commission Regulation (EC) No. 847/2000.

Outcome

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

Loargys is indicated for the treatment of arginase 1 deficiency (ARG1-D), also known as hyperargininemia, in adults, adolescents and children aged 2 years and older.

The CHMP therefore recommends the granting of the marketing authorisation under exceptional circumstances subject to the following conditions:

Conditions or restrictions regarding supply and use

Medicinal product subject to restricted medical prescription (see Annex I: Summary of Product Characteristics, section 4.2).

Other conditions and requirements of the marketing authorisation

- **Periodic Safety Update Reports**

The requirements for submission of periodic safety update reports for this medicinal product are set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and any subsequent updates published on the European medicines web-portal.

The marketing authorisation holder shall submit the first periodic safety update report for this product within 6 months following authorisation.

Conditions or restrictions with regard to the safe and effective use of the medicinal product

- **Risk Management Plan (RMP)**

The marketing authorisation holder (MAH) shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the marketing authorisation and

any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.

- **Additional risk minimisation measures**

Prior to launch of Loargys 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 educational programme is aimed to provide instructions to non-healthcare professionals (patients and caregivers) for proper administration techniques to address the potential risk of medication errors as well as to minimize the potential risk of severe hypersensitivity reaction.

The MAH shall ensure that in each Member State where Loargys is marketed, all patients or caregivers who are expected to administer Loargys as a subcutaneous injection in the home-setting are provided with the following educational material:

- Injection guide for patients and caregivers

This educational material, for patients and caregivers, shall contain the following key messages:

- Instructions on importance of proper handling, preparation and administration of Loargys to reduce the risk of medication errors.
- A detailed description on how to prepare and administer Loargys.
- A description of the signs and symptoms of severe hypersensitivity reactions.
- A description of the recommended course of action if signs and symptoms of hypersensitivity occur.
- Information on the importance of reporting of side effects including hypersensitivity and medication errors.

- **Obligation to conduct post-authorisation measures**

The MAH shall complete, within the stated timeframe, the below measures:

Specific Obligation to complete post-authorisation measures for the marketing authorisation under exceptional circumstances

This being an approval under exceptional circumstances and pursuant to Article 14(8) of Regulation (EC) No 726/2004, the MAH shall conduct, within the stated timeframe, the following measures:

Description	**Due date**
Post-authorisation efficacy study (PAES): in order to collect information on the long- term effectiveness/clinical outcomes in patients with arginase 1 deficiency (ARG1-D) treated with pegzilarginase, the MAH should conduct and submit the results of a study in patients, based on data from a registry.	Annually (with Annual re-assessment)
Non-interventional Post-authorisation safety study (PASS): In order to further characterise the long-term safety and efficacy of pegzilarginase, the MAH should conduct and submit the results of a study in patients with arginase 1 deficiency	Annually (with annual re-assessment)

Description	Due date
(ARG1-D) based on data from a registry.	
In order to further characterise the long-term efficacy and safety of pegzilarginase, the MAH should submit the final results of study CAEB1102-300A, a Phase 3, randomised, double-blind, placebo-controlled study of the efficacy and safety of pegzilarginase in adults, adolescents and children with arginase 1 deficiency (ARG1-D).	31 March 2024
In order to further characterise the long-term efficacy and safety of pegzilarginase, the MAH should submit the final results of study CAEB1102-102A, an open-label extension study to evaluate the long-term safety, tolerability, and efficacy of pegzilarginase in adults, adolescents and children with arginase 1 deficiency (ARG1-D).	31 March 2024
In order to ensure adequate monitoring of safety and efficacy of pegzilarginase in the treatment of arginase 1 deficiency (ARG1-D) in adults, adolescents and children, the MAH shall provide yearly updates on any new information concerning the safety and efficacy of pegzilarginase.	Annually (with Annual re-assessment)

Conditions or restrictions with regard to the safe and effective use of the medicinal product to be implemented by the Member States

Not applicable.

New Active Substance Status

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

Paediatric Data

Furthermore, the CHMP reviewed the available paediatric data of studies subject to the agreed Paediatric Investigation Plan P/0252/2020 and the results of these studies are reflected in the Summary of Product Characteristics (SmPC) and, as appropriate, the Package Leaflet.