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Official Title

A Phase 3 Open-Label Study of Safety, Pharmacokinetics, and Activity of Weekly Subcutaneous Pegzilarginase in Subjects <24 Months Old With Arginase 1 Deficiency

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CLINICAL STUDY PROTOCOL

CAEB1102-301A

A Phase 3 Open-Label Study of Safety, Pharmacokinetics, and Activity of Weekly Subcutaneous Pegzilarginase in Subjects <24 months old with Arginase 1 Deficiency

Sponsor	Immedica Pharma AB Solnavägen 3H 113 63 Stockholm Sweden
EU Clinical Trial Number	2024-510797-25
Protocol Version	2.0
Protocol Date	May 31, 2024

REVISION CHRONOLOGY

- Original version: Version 1.0, dated 29-Feb-2024
- Protocol Version 2.0, dated 31-May-2024

  Changes introduced in Version 2.0 as compared to Version 1.0 are denoted by section below, with deleted text indicated by ~~strikethrough~~ and added text denoted by **bold**:

  - SYNOPSIS and Section 4, Study administrative structure
    - Additional countries for the study to be conducted in: **The Kingdom of Saudi Arabia (KSA). Additional countries may be added if a sufficient number of eligible subjects are not identified in currently planned countries**.
      - Reason for change: the pool of potential subjects for recruitment is limited and new patients identified may be potentially eligible patients.
  - SYNOPSIS and Section 8.1, Inclusion criteria
    - Eligibility criteria, inclusion criteria: **Subjects must weigh > 8 kg due to clinical trial related blood collection volumes required.**
      - Reason for change: Given the pediatric population, the smallest children would not be eligible due to the clinical trial related blood volume requirements.
  - Section 5.1.1, ARG1-D disease-modifying treatment
    - In Studies 102A and 300A, ~~34~~ **44** subjects received SC dosing with pegzilarginase. ~~Three~~ **Six** subjects (~~8.8~~ **13.6**%) reported injection site reactions (ISRs) considered related to study drug: Injection site erythema; Injection site swelling, ~~and~~ Injection site rash, **Injection site pain and Injection site irritation**. The events occurred within 1 to 7 days of dosing. All ISRs were mild in severity, resolved spontaneously or resolved with standard medical care. No subjects discontinued pegzilarginase due to events, nor did they require dose interruption or dose reduction.
      - Reason for change: New long-term extension data became available with an increased frequency of ISRs reported.
  - Section 9.4, Initial Dosing and Dose Modification
    - At Visit 5 and Visit 9, dose modifications will be made if required, based on plasma arginine values (dependent on availability of arginine levels).
      - For Visit 5: review arginine levels 168 hours post doses ~~1~~ **2** and 3 (corresponding to arginine samples taken at Visit ~~2~~ **3** and Visit 4)
      - For Visit 9: review arginine levels 168 hours post doses 5 and 7 (corresponding to arginine samples taken at Visit 6 and Visit 8)
      - Reason for change: Correction of last two samples taken prior to Visit 5.
  - Editorial changes

SIGNATURE PAGE

Sponsor's Approval

Sponsor's Authorized Officer:

[redacted]
Global Medical Head of Genetic and Metabolic Diseases
Immedica Pharma AB
Solnavägen 3H
113 63 Stockholm
Sweden

Sponsor's Signatories:

_____ _____

[redacted] Date

Global Medical Head of Genetic and
Metabolic Diseases, Global Medical Director

INVESTIGATOR'S AGREEMENT

I have read the CAEB1102-301A protocol and agree to conduct the study as outlined. I agree to maintain the confidentiality of all information received or developed in connection with this protocol.

_____

Printed Name of Principal Investigator

_____

Signature of Principal Investigator

_____

Date

1. SYNOPSIS

Name of Sponsor/Company: Immedica Pharma AB		
Name of Investigational Product: Loargys		
Name of Active Ingredient: Pegzilarginase		
Protocol Number: CAEB1102-301A	**Phase:** 3	**Countries:** European Union (EU), United Kingdom (UK), Kingdom of Saudi Arabia (KSA)
Title of Study: A Phase 3 Open-Label Study of Safety, Pharmacokinetics**,** and Activity of Weekly Subcutaneous Pegzilarginase in Subjects < 24 months old with Arginase 1 Deficiency		
Study Centre(s): 3 to 4 sites are planned in the EU, UK and KSA		

Objectives:

Primary Objective:

- To evaluate the effect of pegzilarginase on plasma arginine concentrations in subjects <24 months of age with arginase 1 deficiency (ARG1-D)

Secondary Objectives:

- To evaluate the safety of pegzilarginase
- To characterize the pharmacokinetic (PK) profile of pegzilarginase
- To evaluate the pharmacodynamic (PD) response of pegzilarginase
- To describe changes in physical function

Endpoints:

Primary Endpoint:

- Change from baseline in plasma arginine after 12 weeks of pegzilarginase treatment

Secondary Endpoints:

- Safety assessments will include:
  - adverse events (AEs), including hypersensitivity reactions (HSRs), injection site reactions (ISRs), and hyperammonaemic events
  - laboratory tests, including occurrence of prolonged hypoargininaemia
  - vital signs, physical examinations, growth assessments, and electrocardiograms (ECGs)
- PK parameters evaluation including half-life ($T_{½}$), time to maximum observed concentration (T_{max}), maximum observed concentration (C_{max}), area under the plasma drug concentration-time curve from time 0 to time t (AUC_{0-t}), area under the plasma drug concentration-time curve from time 0 extrapolated to infinite time ($AUC_{0-\infty}$),

extravascular clearance (CL/F), and apparent volume of distribution at steady state after non-intravenous administration (V_{ss}/F)

- PD response evaluation including anti-drug antibodies (ADAs), levels of plasma arginine and ornithine
- Changes in physical function after 12 weeks of pegzilarginase treatment as measured by Gross Motor Function Measure (GMFM)-66 Parts A through E, as age appropriate and feasible

Study Design: This is an open-label, multicentre study to evaluate the safety, PK, and activity (PD) of weekly subcutaneous (SC) administration of pegzilarginase in subjects with ARG1-D who are < 24 months of age. The study consists of a screening period of up to 4 weeks, a subsequent 12-week treatment period, and a safety follow-up period of 8 weeks.

Study Methods: Parent and/or legal guardian of participating subjects must provide written informed consent.

- All subjects will receive a once weekly (QW) SC dose of pegzilarginase for 12 weeks. Subjects will receive their doses at the investigational site to monitor tolerability and assess safety until the Principal Investigator confirms at-home dosing by trained home healthcare personnel is safe and appropriate.
- Subjects will continue to receive a stable diet (protein restriction, +/- essential amino acids [EAAs]) and continue the use of ammonia scavengers, if prescribed.
- Following the completion of treatment, all subjects will have the Visit 13 safety and efficacy follow-up 1 week after the last dose and the Visit 14 safety follow-up visit at the end of 8 weeks

Sample Size Justification: The sample size is solely based on clinical considerations and the EU Paediatric Investigational Plan

Number of Subjects (Planned): 3 subjects

Target Population: Male and female subjects from birth to < 24 months of age with ARG1-D.

Eligibility Criteria:

Subjects are eligible to be included in the study if they meet all of the following criteria:

Inclusion Criteria:

1. Subjects must be < 24 months of age on the date of informed consent
2. Confirmed diagnosis of ARG1-D documented in medical records by at least 1 of the following methods:
    a. elevated plasma arginine levels
    b. a mutation analysis revealing a pathogenic variant
    c. red blood cell (RBC) arginase activity
3. Subjects must weigh > 8 kg due to clinical trial related blood collection volumes required

4. Written informed consent by parent/legal guardian, in accordance with national stipulations, which includes compliance with the requirements and restrictions listed in the informed consent form and in this protocol
5. At least one value of plasma arginine ≥ 180 μM during screening
6. Documented confirmation from the Investigator and/or dietitian that the subject can:
    a. attempt to maintain a stable, age-appropriate level of protein consumption, including natural protein, and EAA supplementation within approximately ± 15% of dietitian recommended diet
    b. attempt to maintain current use of ammonia scavengers, if prescribed

Subjects are excluded from the study if any of the following criteria are met:

Exclusion Criteria:

1. Other medical condition(s) or comorbidity(ies) that, in the opinion of the Investigator, would interfere with study compliance or data interpretation
2. Hyperammonaemic episode (plasma ammonia levels > 100 μM) with ≥ 1 symptom related to hyperammonaemia requiring hospitalisation or emergency room management within the 4 weeks before the first dose of study drug
3. Active infection requiring anti-infective therapy within < 2 weeks before first dose of study drug
4. Known active infection with human immunodeficiency virus, hepatitis B, or hepatitis C
5. History of hypersensitivity to polyethylene glycol (PEG) or any of the excipients included in the study drug that, in the judgment of the Investigator, puts the subject at unacceptable risk for AEs
6. Currently participating in another therapeutic clinical study or has received any investigational agent within 30 days (or 5 half-lives, whichever is longer) prior to first dose of study drug
7. Previous liver or haematopoietic stem cell transplant
8. Use of botulinum toxin within 16 weeks prior to first dose

Screen Failures: Subjects who do not meet eligibility criteria (screen failure) may be re-screened a maximum of 2 times after the initial screening, for a total of 3 screenings

Study Drug, Dosage and Mode of Administration:

Study Drug

Loargys (pegzilarginase) is a cobalt (Co^{2+})-substituted, pegylated, recombinant human arginase 1.

Medicinal product in the strength of 5 mg/mL will be used in this study.

Route of Administration

Study drug will be administered QW via SC injection.

Dose

The starting dose will be 0.1 mg/kg per week with the ability to adjust the dose in 0.05 mg/kg increments between 0.05 mg and 0.2 mg/kg to maintain pre-dose arginine level in the predefined range of 50 - 150 μM

Duration of Study Participation: The total study duration is approximately 24 weeks consisting of:

- Up to 4 weeks screening
- 12 weeks active treatment
- 8 weeks follow-up

Study Assessments

Safety Assessments:

Safety will be monitored during the trial with recording of AEs and concomitant medications, routine laboratory assessments, physical examinations, growth assessments, ECGs, vital signs, and ADA when available. HSRs, ISRs, and hyperammonaemic episodes will be closely monitored.

Safety Review Committee (SRC) and Safety Reviews:

A formal SRC is not planned for this study. Safety will be monitored by the Sponsor and the study Investigators.

Laboratory Assessments:

- PK samples for pegzilarginase
- PD assessments for pegzilarginase: arginine and ornithine
- Ammonia
- Routine safety laboratory tests
    - Haematology (complete blood count including RBC, white blood cell with differential count, haemoglobin, haematocrit, and platelet count)
    - Serum chemistry (sodium, potassium, chloride, blood urea nitrogen, creatinine, total bilirubin, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and lactate dehydrogenase)
    - Coagulation (prothrombin time, partial thromboplastin time, and international normalized ratio)
- ADAs

Other Assessments:

- Vital signs
- Weight, physical examinations, and growth assessments
- 12-lead ECGs
- Neuromotor GMFM-66 Parts A through E, as age appropriate and feasible

- Individualised diet management questionnaire
- 3-Day diary diet record

Reference Therapy, Dosage and Mode of Administration: Not applicable

Withdrawal/Discontinuation of Treatment:

A subject may withdraw from the study treatment and/or study at any time at the parent/legal guardian's request or may be withdrawn at any time at the discretion of the Investigator or Sponsor for any reason. Any circumstance for early withdrawal will be deemed an "early termination" if all visits have not been completed. Reason(s) for early termination identified by the Principal Investigator will be captured in the medical record and the case report form. Specific reasons for early termination may include, but are not restricted to, the following:

- Withdrawal of consent
- Investigator decision
- Concurrent illness
- AE
- Significant protocol deviation or non-compliance
- Termination of the study by the Sponsor
- Lost to follow-up

If the subject's parent/legal guardian withdraws consent, the Sponsor may retain and continue to use any data collected before such withdrawal of consent, but there will be no further visits or data collected.

If a subject prematurely discontinues study treatment, they may need to be replaced based on Sponsor's assessment on completeness of subject's data.

Pharmacokinetic Analyses:

PK samples will be collected at the following timepoints:

- Pre-dose at Visits 1, 2, 4, 6, 8, and 10
- Between 24- and 48-hours post-dose at Visits 2, 4, and 10
- Visit 13 (168-hours post dose 12)
- If there are blood draws for other reasons, an extra tube should be collected, if possible, for PK analysis at the same time (if permitted by total daily volume limits), and the time relative to dosing will be recorded.

PK parameters to be evaluated are:

- $T_{½}$
- T_{max}
- C_{max}
- AUC_{0-t}
- $AUC_{0-\infty}$
- CL/F
- V_{ss}/F

Statistical Analyses:

Endpoints and other assessments will be presented using descriptive statistics only.

No interim analysis is planned for this study.

2. TABLE OF CONTENTS

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3. LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation or Special Term	Explanation
ADA(s)	anti-drug antibodies
AE	adverse event
ARG1	arginase 1
ARG1-D	arginase 1 deficiency
AUC_{0-t}	area under the plasma drug concentration-time curve from time 0 to time t
$AUC_{0-\infty}$	area under the plasma drug concentration-time curve from time 0 extrapolated to infinite time
BT	botulinum toxin
CDC	centers for disease control and prevention
CL	clearance
CL/F	extravascular clearance
C_{max}	maximum observed concentration
Co^{2+}	cobalt
Co-Arg1-PEG	pegzilarginase drug substance
eCRF	electronic case report form
CRO	contract research organization
EAA	essential amino acid
EC	enzyme commission
ECG	electrocardiogram
EMA	European medicines agency
ET	early termination
EU	European union
F	bioavailability
GCP	good clinical practice
GMFM	gross motor function measure
HA	hyperammonaemia
HSR	hypersensitivity reaction
ICF	informed consent form
ICH	international council for harmonisation of technical requirements for pharmaceuticals for human use
ID	identification

Abbreviation or Special Term	Explanation
IDM	individualised disease management
IEC	independent ethics committee
INR	international normalised ratio
ISR	injection site reaction
IV	intravenous(ly)
LTE	long-term extension
MedDRA	medical dictionary for regulatory activities
Mn^{2+}	manganese
mPEG	monomethoxy polyethylene glycol
PD	pharmacodynamic(s)
PEG	polyethylene glycol
PK	pharmacokinetic(s)
PT	prothrombin time
PTT	partial prothrombin time
QW	once weekly
RBC	red blood cell
SAE	serious adverse event
SAP	statistical analysis plan
SC	subcutaneous(ly)
SFU	safety follow-up
SOC	system organ class
SRC	safety review committee
$T_{½}$	half-life
TEAE	treatment-emergent adverse event
T_{max}	time to maximum observed concentration
ULN	upper limit of the normal range
US	United states
UK	United kingdom
V_{ss}	volume at steady state
V_{ss}/F	apparent volume of distribution at steady state after non-intravenous administration

4. STUDY ADMINISTRATIVE STRUCTURE

This study is planned to be conducted at 3 to 4 sites in the European Union (EU), United Kingdom (UK) and Kingdom of Saudi Arabia (KSA). Additional countries may be added if a sufficient number of eligible subjects are not identified in currently planned countries.

The name, telephone and fax numbers of the medical monitor and other contact personnel at the Sponsor and study vendors are listed in the regulatory binder provided to each site.

5. INTRODUCTION

5.1. Background

Arginase 1 deficiency (ARG1-D) (Orpha number ORPHA90; ICD-10 code: E72.2; OMIM number 207800) is a rare, progressive, multisystem, autosomal recessive disease (Summar et al., 2013; Diez-Fernandez et al., 2018; Schlune et al., 2015; Waisbren et al., 2018).

This disease typically presents in early childhood and is caused by deficiency in the enzyme arginase 1 (ARG1 [Enzyme Commission {EC} 3.5.3.1]), which leads to 2 important harmful metabolic effects:

- Accumulation of high levels of arginine and arginine-derived metabolites.
- Impairment of the urea cycle, which leads to episodic elevation of ammonia levels.

The high plasma arginine level is believed to be the key driver of spasticity, developmental delay, and seizures, which develop in early childhood and progress over time (De Deyn et al., 1997; Waisbren et al., 2018). The lower-limb spasticity in early childhood impairs mobility and balance, leading to difficulties in walking and climbing stairs. School performance and educational achievement is markedly impacted by the developmental delay and the ensuing cognitive decline. The neuromotor and neurocognitive effects, which occur in spite of individualised disease management (IDM) approaches, result from persistently elevated arginine levels that have a profound impact on daily functioning and quality of life from early in life with progressive worsening over time leading to severe disabilities and early death (De Deyn et al., 1997; Oeffinger et al., 2008; Prasad et al., 1997; Carvalho et al., 2012).

In addition to the severe neuromotor and neurocognitive manifestations, which dominate the clinical picture, subjects with ARG1-D manifest other medically important disease-related abnormalities, including complications due to hyperammonaemia (HA), hepatocellular injury (Häberle et al., 2019; Schlune et al., 2015), inadequate nutrition, and growth impairment. These abnormalities are a result of the disease or the current disease management with protein restriction.

The goals for long-term management of patients with ARG1-D are to reduce plasma arginine levels sufficiently to prevent development and progression of clinical manifestations without adversely impacting normal growth and development and to prevent HA. Recommended disease management involves a common framework of protein restriction and essential amino acid (EAA) supplementation and/or use of pharmacological treatments for any disease manifestations, including ammonia scavengers. In isolation, management of arginine levels is complicated by the impairment of the urea cycle and the need to ensure an adequate intake of protein for proper growth and development while avoiding HA. Disease management is individualised with titration of different components to aim for optimal control of plasma arginine levels, adequate protein intake for proper growth and development, and prevention of HA.

Disease management with dietary protein restriction has been shown to lower plasma arginine levels in some subjects, with amelioration of some of the disease-related abnormalities, thus providing support for the value of arginine reduction. However, this approach is inadequate in most subjects as demonstrated by the persistence of the marked hyperargininaemia, mainly due to the dietary restrictions not targeting endogenous arginine production. Moreover, the diet is

difficult to maintain and manage, especially in growing children, and requires supplementation with unpalatable EAA formula to maintain a safe amino acid intake (Häberle et al., 2019; Huemer et al., 2016; Lambert et al., 1991; Burrage et al, 2015). Liver transplantation has been reported to achieve disease normalisation in some subjects, but this intervention is available to only a small fraction of subjects and carries substantial additional risks.

5.1.1. ARG1-D disease-modifying treatment

Loargys (pegzilarginase) is a cobalt (Co^{2+}) substituted, recombinant human ARG1 enzyme that is covalently conjugated to monomethoxy polyethylene glycol (mPEG). The enzyme is expressed as a recombinant protein in *Escherichia coli* cells and metabolises arginine. Human ARG1 is a binuclear manganese (Mn^{2+}) metalloenzyme that catalyses the hydrolysis of arginine to yield ornithine and urea. Pegzilarginase substitutes for the deficient ARG1 enzyme in patients with ARG1-D by providing an alternate pathway for arginine breakdown in the plasma via the enzymatic conversion of arginine to its natural metabolic product ornithine.

Loargys was granted a marketing authorisation in the EU and Great Britain for the treatment of ARG1-D in adults, adolescents and children aged 2 years and older in December 2023.

Biological activity of pegzilarginase has been demonstrated based on non-clinical studies and clinical data from the completed clinical development program, consisting of 3 clinical studies, in which a total of 48 subjects received pegzilarginase treatment:

- Study CAEB1102-101A (Study 101A), a Phase 1/2, open-label, 2-part (Part 1 [single ascending-dose escalation] and Part 2 [repeated dosing]) study to investigate the safety, pharmacokinetics (PK), and pharmacodynamics (PD) of IV pegzilarginase in 16 paediatric and adult subjects with ARG1-D;
- Study CAEB1102-102A (Study 102A), a Phase 2, open-label, LTE study to evaluate the long-term safety, tolerability, and efficacy of IV and SC pegzilarginase in 14 subjects with ARG1-D who completed Part 2 of Study 101A;
- Study CAEB1102-300A (Study 300A), a Phase 3, randomized study consisting of a 24-week, double-blind (DB), placebo-controlled period followed by an open-label, long-term extension (LTE) period to evaluate the efficacy and safety of intravenous (IV) and subcutaneous (SC) pegzilarginase in 32 paediatric and adult subjects with ARG1-D

The pivotal phase 3 Study 300A met its primary endpoint, demonstrating both a clinically and statistically significant reduction in plasma arginine with pegzilarginase compared to placebo (estimated reduction relative to placebo: 76.7 %, $p < 0.0001$) after 24 weeks of treatment. Plasma arginine levels below guideline recommended target and within normal range were achieved in 90.5 % of pegzilarginase-treated subjects compared to 0 % of the subjects in the placebo arm ($p < 0.0001$). Further, pegzilarginase demonstrated clinically meaningful improvements in mobility as assessed by neuromotor function during the 24-week double-blind period of Study 300A.

In the long-term extension phases of studies 102A and 300A, subjects could receive once weekly (QW) SC administration of pegzilarginase. Doses from IV to SC were to remain the same unless the dosing algorithm dictated otherwise. SC dosing demonstrated continued maintenance of 168-hour post-dose arginine levels, similar to those achieved after IV administration. The clinically meaningful improvements in mobility as assessed by neuromotor function that were seen after 24

weeks were maintained or continued to improve through 96 weeks of treatment in Study 102A. These results are especially clinically relevant in a population that would be expected to experience detectable disease progression, including decreased mobility, over time.

Data from the Study 300A demonstrated the median time to maximum observed concentration (T_{max}) generally occurred rapidly (≤ 4.7 hours after the start of infusion), as expected following a nominal 0.5 hours QW IV infusion of pegzilarginase. The pegzilarginase pharmacokinetic (PK) exposures (maximum observed concentration [C_{max}] and area under the plasma drug concentration versus time curve from time 0 to 168 hours) after IV administration increased in an approximately dose-proportional manner across the dose range of 0.05 to 0.2 mg/kg at steady state after repeat QW dosing (Week 12 and Week 24). The mean half-life ($T_{½}$) was approximately 40 hours (range: 37.3 to 43 hours), which was similar across doses and whether a single dose or at steady state. Steady state was achieved on or before Week 12 based on the available data and sampling time; however, it is theoretically expected to be reached after 2 weeks of consistent QW dosing based on $T_{½}$.

Anti-drug antibodies (ADAs) were transient and generally low in titre: titres higher than 1:40 were observed in 2 of 8 subjects with anti-pegzilarginase ADAs and titres higher than 1:50 were observed in 1 of 2 subjects with anti-polyethylene glycol (PEG) ADAs. Low titre ADAs had no impact on PK and pharmacodynamics (PD) (based on available data). The effect of the anti-PEG ADAs with titre 1:800 on PK in 1 subject could not be further evaluated due to unavailability of additional PK data; however, although arginine levels were reduced after the initial doses, arginine levels increased toward baseline levels starting the week prior to testing ADA positive.

Hypersensitivity Reactions (HSRs) are known effects of biologic therapy. During the clinical studies, 6 subjects (12.5%) experienced signs and symptoms either consistent with, or that may be related to a HSR when administered IV. Hypersensitivity occurred following at least 1 previous dose of pegzilarginase and began soon after initiation of the IV infusion. HSRs were generally mild or moderate, transient, and did not require dose reduction or discontinuation. Observed signs and symptoms included rash, facial swelling, feeling hot and flushed, shivering, cough, dyspnoea and abdominal pain. HSRs were transient and were managed by temporarily stopping or slowing the infusion and administering medication (e.g., antihistamines, corticosteroids and in some cases also antipyretics). No subject discontinued treatment due to an HSR. All events of hypersensitivity resolved, enabling completion of pegzilarginase infusion on the same day.
In Studies 102A and 300A, 44 subjects received SC dosing with pegzilarginase. Six subjects (13.6%) reported injection site reactions (ISRs) considered related to study drug: Injection site erythema; Injection site swelling, Injection site rash, Injection site pain and Injection site irritation. The events occurred within 1 to 7 days of dosing. All ISRs were mild in severity, resolved spontaneously or resolved with standard medical care. No subjects discontinued pegzilarginase due to events, nor did they require dose interruption or dose reduction.

For further details, refer to the approved Summary of Product Characteristics (SmPC) for Loargys.

5.2. Study Rationale

Subjects with ARG1-D show persistent hyperargininaemia and continued disease progression despite current IDM consisting of severe protein restriction and EAA supplementation and/or

ammonia scavengers (Huemer et al., 2016). The failure to adequately lower plasma arginine levels with IDM into the normal range, or even below the current guideline-recommended level of 200 µM (Häberle et al., 2019) in most cases, is believed to be due to both the practical challenges of adhering to a protein-restricted diet rigorous enough to lower plasma arginine levels and the important contribution of whole-body protein turnover to plasma arginine flux (Häberle et al., 2019; Huemer et al., 2016; Lambert et al., 1991; Oeffinger et al., 2008; Prasad et al., 1997; Wu and Morris 1998).

Pegzilarginase has been shown in previous studies to produce marked, rapid, and sustained reductions in plasma arginine levels in subjects aged 2 years and above with ARG1-D, allowing substantially improved arginine control relative to what can be achieved with IDM approaches. The improved control of plasma arginine levels was accompanied by clinical improvements in one or more instruments of neuromotor function, consistent with the hypothesis that improved plasma arginine control has the potential to slow disease progression in affected patients.

The current study seeks to evaluate the safety, PK, and activity of subcutaneous pegzilarginase for the treatment of ARG1-D in subjects < 24 months old.

5.3. Benefit-Risk Assessment

As discussed in Section 5.1, ARG1-D is a rare, serious, progressive disease with significant unmet need despite IDM approaches. The disease typically presents in childhood with serious neuromotor and neurocognitive manifestations that progress with increasing age with resultant severe disabilities, liver function abnormalities, HA, significant ill health, and a shortened life expectancy.

Clinical experience with pegzilarginase in both adult and paediatric subjects > 2 years of age with ARG1-D have shown that pegzilarginase has a tolerability profile that supports IV and SC administered doses up to 0.2 mg/kg. Treatment-emergent adverse events (TEAEs) in subjects with ARG1-D have generally been mild or moderate.

Data from the completed clinical development program have demonstrated that pegzilarginase produces marked and sustained reduction of plasma arginine levels in subjects with ARG1-D. The improved control of plasma arginine levels was accompanied by clinically relevant treatment effects in neuromotor function.

Adverse events (AEs) associated with administration of other approved biologic enzyme therapies include hypersensitivity reactions (HSRs). HSRs have been reported with pegzilarginase administered IV, but not SC, and have been manageable with standard medical care. Given the potential for HSRs with administration of biologics, measures have been incorporated into this protocol to minimize risk while monitoring subject safety. Reactions requiring intervention are typically managed by standard medical care.

Injection Site Reactions (ISRs) are also known to be associated with biologic therapies. ISRs have been reported with pegzilarginase and were self-limited or managed with standard medical care.

Risk minimization procedures are implemented for this study to minimize risks to the subjects. Specific eligibility criteria will ensure that subjects who present with characteristics that may increase the risk for an adverse outcome are excluded. The occurrence of AEs will be monitored

throughout the study. Since this study involve paediatric subjects, blood sampling (volumes and occasions) will be strictly controlled in accordance with applicable guidelines, and the study participants will receive standard of care at the site by trained professionals, if needed.

Overall, the benefit-risk assessment for pegzilarginase is considered favourable in the study population of paediatric (< 24 months) subjects with ARG1-D, given the:

- Acceptable tolerability and manageable risk profile in paediatric (age ≥ 2 years) and adult subjects in previous clinical trials
- Rapid reduction of plasma arginine levels
- Clinical improvements in neuromotor manifestations.

6. OBJECTIVES AND ENDPOINTS

Objectives:

Primary Objective:

- To evaluate the effect of pegzilarginase on plasma arginine concentrations in subjects <24 months of age with ARG1-D

Secondary Objectives:

- To evaluate the safety of pegzilarginase
- To characterise the PK profile of pegzilarginase
- To evaluate the PD response of pegzilarginase
- To describe changes in physical function

Endpoints:

Primary Endpoint:

- Change from baseline in plasma arginine after 12 weeks of pegzilarginase treatment

Secondary Endpoints:

- Safety assessments will include:
  - AEs, including HSRs, ISRs, and hyperammonaemic events
  - Clinical laboratory tests (chemistry, haematology, coagulation), plasma ammonia and occurrence of prolonged hypoargininaemia
  - vital signs, physical examinations, growth assessments, and electrocardiograms (ECGs)
- PK parameters evaluation including $T_{½}$, T_{max}, C_{max}, area under the plasma drug concentration-time curve from time 0 to time t (AUC_{0-t}), area under the plasma drug concentration-time curve from time 0 extrapolated to infinite time ($AUC_{0-\infty}$), extravascular clearance (CL/F), and apparent volume of distribution at steady state after non-intravenous administration (V_{ss}/F)
- PD response evaluation: ADAs, levels of plasma arginine and plasma ornithine
- Changes in physical function after 12 weeks of pegzilarginase treatment as measured by Gross Motor Function Measure (GMFM)-66 Parts A through E, as age appropriate and feasible

7. INVESTIGATIONAL PLAN

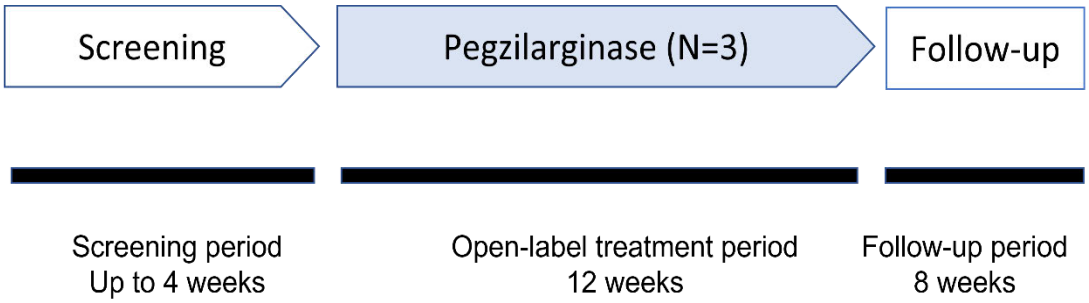
7.1. Overall Study Design and Plan

CAEB1102-301A is an open-label, single-arm, non-controlled, repeat dosing, multicentre study to evaluate the safety, PK, and activity (PD) of weekly SC administration of pegzilarginase over 12 weeks in subjects with ARG1-D who are < 24 months of age. This study will consist of:

- A screening period of up to 4 weeks to ensure the subjects meet the study eligibility criteria and establish baseline plasma arginine
- A treatment period of 12 weeks
- A safety follow-up period of 8 weeks with visits 1 week and 8 weeks after the last dose.

The schema of the study is provided in Figure 1.

Figure 1: Study Schema for CAEB1102-301A



At Screening, the parent and/or legal guardian of the participating subjects must provide written informed consent.

After inclusion in the study, all subjects will receive a QW SC dose starting at 0.1 mg/kg pegzilarginase for 12 weeks with the ability to adjust the dose in 0.05 mg/kg increments between 0.05 mg and 0.2 mg/kg to maintain pre-dose arginine level in the predefined range of 50 - 150 µM. Subjects will receive their doses at the investigational site to monitor tolerability and assess safety until the Principal Investigator and Sponsor confirm at-home dosing is safe and appropriate by trained home healthcare personnel.

Subjects will continue to receive a stable diet (protein restriction, +/- EAA) and continue the use of ammonia scavengers, if prescribed.

Following the completion of treatment, all subjects will have the Visit 13 efficacy and safety follow-up after 1 week and the Visit 14 safety follow-up (SFU) visit at the end of 8 weeks.

7.2. Scientific Rationale for Study Design

The study design is employed to demonstrate the safety, PK, and activity (PD) of pegzilarginase in subjects < 24 months of age with ARG1-D in accordance with the EU Paediatric Investigational Plan. Pegzilarginase has been demonstrated to produce marked, rapid, and

sustained reductions in plasma arginine levels in subjects aged 2 years and above with ARG1-D, allowing substantially improved arginine control relative to what can be achieved with IDM approaches. The improved control of plasma arginine levels was accompanied by clinical improvements in one or more instruments of neuromotor function, consistent with the hypothesis that improved plasma arginine control has the potential to slow disease progression in affected patients. This study serves to provide additional safety, PK, and activity (PD) data in the paediatric population < 24 months of age to the data captured in the completed pegzilarginase clinical development program in patients aged 2 years and above with ARG1-D.

The length of the study is deemed appropriate to evaluate the effect of pegzilarginase on plasma arginine concentrations and its safety and PK in subjects < 24 months of age with ARG1-D. The study duration is in alignment with the design in the agreed Paediatric Investigational Plan for the European Medicines Agency (EMA).

7.2.1. Study Population

Early intervention in paediatric patients with ARG1-D, prior to the development of significant neuromotor, adaptive behaviour and neurocognitive manifestations, offers the potential to halt the progression or facilitate clinical improvement; therefore, treatment of subjects < 24 months of age is justified based on the data from earlier clinical trials supporting the potential for benefit in this age group.

7.3. Justification for Dose

The dose selection was derived using a population PK-PD model developed with PK and PD data from previous studies in the pegzilarginase clinical development program, which included data from patients > 2 years of age. A starting dose of 0.1 mg/kg SC is expected to provide adequate control of arginine prior to dose titrations in a majority of the subjects aged < 24 months. This starting dose combined with a dose increment of 0.05 mg/kg is expected to yield the optimal control of arginine levels over the dosing interval, i.e., minimising the time that arginine remains below the lower limit of the normal and maximising the time that arginine remains in the predefined normal range.

7.4. Definitions of End of Treatment and Start and End of Study

7.4.1. End of Treatment

The end of treatment for an individual subject is defined as the date of the last dose at Visit 12 (Appendix 1, Table 4) or the date of their last dose if they discontinue prematurely.

7.4.2. Start and End of Study

The start of the study is defined as the first act of recruitment of a potential subject for this study. The start of the study for an individual subject is defined as the signing of the informed consent form.

The end of study for the study overall is defined as the last subject visit at any participating site. The end of the study for an individual subject is defined as the date when the last study assessment for this subject has been completed.

Subjects who successfully complete the study and demonstrate benefit of treatment (reduction of plasma arginine levels) will be offered expanded access to commercially available Loargys outside the clinical trial until the subject is 2 years of age or the indication for Loargys is extended to include patients below 2 years of age, whichever occurs first. Should any findings from this study indicate that treatment with Loargys in subjects below 2 years of age is not suitable, treatment will be stopped. During continued treatment the subjects will be managed and followed up in accordance with standard clinical care, and safety data will be reported in accordance with applicable regulations. The treating physician will be responsible for securing any applicable ethical approvals and parental consents according to national regulations. No further data will be collected during the continued treatment. All Investigators are encouraged to enrol their subjects into existing disease registries after completion of the studies.

8. STUDY POPULATION

8.1. Inclusion Criteria

1. Subjects must be < 24 months of age on the date of informed consent
2. Confirmed diagnosis of ARG1-D documented in medical records by at least 1 of the following methods:
    a. elevated plasma arginine levels
    b. a mutation analysis revealing in a pathogenic variant
    c. red blood cell (RBC) arginase activity
3. Subjects must weigh > 8 kg due to clinical trial related blood collection volumes required
4. Written informed consent by parent/legal guardian, in accordance with national stipulations, which includes compliance with the requirements and restrictions listed in the informed consent form (ICF) and in this protocol
5. At least one value of plasma arginine ≥ 180 μM during screening
6. Documented confirmation from the Investigator and/or dietitian that the subject can:
    a. attempt to maintain a stable, age-appropriate level of protein consumption, including natural protein, and EAA supplementation within approximately ±15% of dietitian recommended diet
    b. attempt to maintain current use of ammonia scavengers, if prescribed

8.2. Exclusion Criteria

1. Other medical conditions or comorbidity(ies) that, in the opinion of the Investigator, would interfere with study compliance or data interpretation
2. Hyperammonaemic episode (plasma ammonia levels > 100 μM) with ≥ 1 symptom related to HA requiring hospitalisation or emergency room management within the 4 weeks before the first dose of study drug
3. Active infection requiring anti-infective therapy within < 2 weeks before first dose of study drug
4. Known active infection with human immunodeficiency virus, hepatitis B, or hepatitis C
5. History of hypersensitivity to PEG or any of the excipients included in the study drug that, in the judgment of the Investigator, puts the subject at unacceptable risk for AEs
6. Currently participating in another therapeutic clinical study or has received any investigational agent within 30 days (or 5 half-lives, whichever is longer) prior to first dose of study drug
7. Previous liver or haematopoietic stem cell transplant
8. Use of botulinum toxin (BT) within 16 weeks prior to first dose

8.3. Screen Failures and Re-Screening

A screen failure is defined as a subject for whom consent to participate in the clinical study was obtained but who was subsequently not included. Information to be collected about screen-failed subjects will be outlined in the electronic case report form (eCRF) completion guidelines.

Subjects who do not meet the eligibility criteria (screen failure) may be re-screened a maximum of 2 times after the initial screening, for a total of 3 screenings.

8.4. Replacement of Subjects

If a subject prematurely discontinues study treatment, they may need to be replaced based on Sponsor's assessment on completeness of subject's data.

9. STUDY MEDICATION

9.1. Study Medication Characteristics

Table 1: Loargys Characteristics

Intervention Name	Loargys (pegzilarginase)
Unit Dose Strength(s)	5 mg/mL
Description	Pegzilarginase is a cobalt-substituted, pegylated, recombinant human Arginase 1. Pegzilarginase drug product is supplied as a liquid formulation in 3 mL single-use glass vials containing 0.4 mL of formulated drug product at a concentration of 5 mg/mL, i.e. 2 mg of pegzilarginase.
Sourcing	Provided by Sponsor or sourced by the sites (in this case, costs will be reimbursed by the Sponsor)
Marketing authorization	Great Britain: PLGB 53487/0007 EU: EU/1/23/1774/001
Packaging and Labelling	Study medication will be the commercialized product that is provided in a single-use glass vial containing 0.4 mL of drug product solution. Each vial or vial carton will be labelled as required per country requirement.

Loargys is currently authorized in the EU and Great Britain for the treatment of ARG1-D in patients 2 years and older. In this study, it will be used outside the terms of these marketing authorizations in subjects younger than 2 years of age.

9.2. Storage/Preparation/Accountability

Storage: Loargys should be stored in a refrigerator (2 °C - 8 °C). Do not freeze. Store in the original carton in order to protect from light. For storage conditions after preparation/dilution of the medicinal product, see SmPC Section 6.3.

Preparation: Details for Loargys preparation are provided in the SmPC.

Accountability: As part of the monitoring procedures for this study, a clinical research associate will perform review of documented drug accountability.

At the end of the study, any remaining medicinal product should be destroyed at the local hospital pharmacy after prior approval by Sponsor. A certificate of destruction should be issued.

9.3. Premedication and Post-dosing Observation

As per clinical judgment, premedication with an antihistamine and/or corticosteroid may be given 30 minutes prior to dosing to reduce the risk of HSRs/ISRs in a subject who experienced a HSR/ISR during previous dosing.

The study drug administrations should be performed under medical observation where proper medical care for hypersensitivity reactions could be provided. Subjects should be observed for a minimum of 1 hour following completion of dose administration.

9.4. Initial Dosing and Dose Modification

Initial Dose Level:

Dosing will begin at Dose Level 1, 0.1 mg/kg weekly for the first 4 weeks (Table 2).

Timing of Dose Modifications:

At Visit 5 and Visit 9, dose modifications will be made if required, based on plasma arginine values (dependent on availability of arginine levels).

- For Visit 5: review arginine levels 168 hours post doses 2 and 3 (corresponding to arginine samples taken at Visit 3 and Visit 4)
- For Visit 9: review arginine levels 168 hours post doses 5 and 7 (corresponding to arginine samples taken at Visit 6 and Visit 8)

Dosing Modification Algorithm:

Any dose modifications required based on plasma arginine levels and/or changes in the subject's clinical presentation will be implemented by the Investigator in discussion with the Sponsor's medical team. Subjects' arginine values should be assessed for the above scheduled potential dose change/adjustment if the following conditions are met:

- If two consecutive arginine results are not available, keep the dose the same and delay the dose adjustment review until two consecutive arginine values are available
- If two consecutive results are available and both plasma arginine levels prior to the dose adjustment visit are > 150 µM, without a prior missed dose, increase the dose by 1 level
- If two consecutive results are available and both plasma arginine levels prior to the dose adjustment visit are < 50 µM, irrespective of any missed doses, decrease the dose by 1 level (refer to Table 2)

Following any dose adjustment, 2 new consecutive arginine levels are required prior to additional dose adjustment. If you are unsure how to proceed regarding a dose adjustment in your subject, please consult the Sponsor prior to making any dose adjustments.

Table 2: Dose Adjustments for Loargys

Dose Level	Dose
-1	0.05 mg/kg
1[a]	**0.10 mg/kg (Starting / Nominal Dose)**
2	0.15 mg/kg
3	0.20 mg/kg

[a] Loargys dosing starts at 0.1 mg/kg. Dose changes are in 0.05 mg/kg increments.

Volume Adjustments

Volume of SC dose should be calculated based on the subject's weight at baseline, unless the current weight has changed by ≥ ± 10 % of the baseline weight. If the subject's weight has changed ≥ ± 10 %, the new weight should be used to calculate the dose volume.

Missed Doses

Missed doses will not be made up for.

Incorrect Doses

If a subject receives an incorrect dose, then the administered dose will be recorded in the eCRF and the site source documentation. The subject will then return to the prescribed dose per the protocol algorithm at the next visit.

9.5. Concomitant Therapy

Ammonia Scavengers

Subjects will be maintained on the ammonia scavenger regimen prescribed prior to enrolment by the treating Investigator throughout the study. The prescribed regimen will not be modified during the study unless clinically indicated in the opinion of the Investigator. Any changes in dose will be recorded in the eCRF.

Other Concomitant Therapy

Any medical intervention, including over-the-counter or prescription medicines, vitamins, dietary supplements, vaccines, or surgery that the subject is receiving at the time of informed consent or during participation in the study must be recorded along with:

- Reason for intervention (e.g., adverse event or medical history)
- Dates of administration including start and end dates
- Dosage information including dose and frequency.

9.6. Prohibited Medications

BT may not be used in the 16 weeks prior to first dose in the study and should not be used during the study until subjects have completed the study unless medically required. If it becomes medically necessary per the Investigator's clinical judgment to utilize BT during the trial, it should be discussed and agreed to with the Sponsor prior to administration, and the dose and location of BT use should remain stable until the end of the study.

10. EARLY TERMINATION

10.1. Discontinuation of Study Medication or Study

A subject may withdraw from the study treatment and/or study at any time at the parent/legal guardian's request or may be withdrawn at any time at the discretion of the Investigator or Sponsor for any reason. Any circumstance for early withdrawal will be deemed an "early termination" if all visits have not been completed. Reason(s) for early termination identified by the Principal Investigator will be captured in the medical record and the eCRF.

Specific reasons for early termination may include, but are not restricted to, the following:

- Parental/legal guardian's withdrawal of consent
- Investigator decision
- Concurrent illness
- AEs
- Significant protocol deviation or non-compliance
- Termination of the study by the Sponsor
- Lost to follow-up.

Subjects who are early terminated for any reason (except for withdrawal of consent) prior to completing all required study visits should complete Visit 13/Early Termination Visit and SFU Visit 14 as specified in the schedule of assessments (Appendix 1, Table 4).

If the subject's parent/legal guardian withdraws consent, the Sponsor may retain and continue to use any data collected before such withdrawal of consent, but no further visits will occur, and no additional data will be collected.

10.2. Lost to Follow-up

A subject will be considered lost to follow-up if they repeatedly fail to return for scheduled visits and the subject's parent/legal guardian is unable to be contacted by the study site.

The following actions must be taken if a subject fails to return to the clinic for a required study visit:

1. The site must attempt to contact the subject's parent/legal guardian and reschedule the missed visit as soon as possible, counsel the subject's parent/legal guardian on the importance of maintaining the assigned visit schedule and ascertain if the parent/legal guardian wishes to and/or will continue the subject in the study.
2. Before a subject is deemed lost to follow-up, the Investigator or designee must make every effort to regain contact with the subject's parent/legal guardian (where possible, 3 telephone calls and, if necessary, a certified letter to the subject's parent/legal guardian's last known mailing address or local equivalent methods). These contact attempts will be documented in the subject's medical records.
3. Should the subject's parent/legal guardian continue to be unreachable, the subject will be considered to have withdrawn from the study.

11. STUDY ASSESSMENTS AND PROCEDURES

11.1. Study Flow and Visit Schedule

The study-specific assessments and procedures are provided in Appendix 1 (Table 4).

11.2. Informed Consent

The subject's parent(s) or legal guardian, as required by local stipulations, will be given an oral explanation of the study, including information about the study drug and the study procedures and will have all questions adequately addressed. It must be emphasized that participation is voluntary, and that the subject's parent(s) or legal guardian have the right to withdraw the subject from the clinical study at any time without prejudice. Written informed consent must be obtained from the subject's parent(s) or legal guardian before any study-related procedures are performed, and if there is a change in study procedures or new safety information becomes available that may affect the parent(s) or legal guardian's willingness to permit the subject's participation in the study. The Investigator must sign and date the informed consent as well.

A copy of the subject information and informed consent form will be given to the parent(s) or legal guardian for their records. The rights and welfare of the subjects will be protected by emphasizing to the parent(s) or legal guardian that the quality of the subject's medical care will not be adversely affected if they decline participation of the subject in this clinical study.

11.3. Demographics and Medical History

Data should be collected and captured in the CRFs including the following:

- Demographic data will include date of birth, sex, age, race, and ethnicity. If local regulations do not allow a full date of birth to be collected, the birth date will be reported to the extent allowed (e.g., month and year or year only)
- Disease characteristics will include age at initial symptoms and details of initial symptoms, method of diagnosis and result, genetic mutation details, motor function abilities, use of assistive devices, and details of specific manifestations (spasticity, muscle cramps, seizures, cognitive/language delay, liver injury/dysfunction)
- Relevant medical and surgical history
- Medications administered in the last 30 days prior to first dose (with the exception of BT, which should be collected for 16 weeks prior to dosing)
- Date of diagnosis and onset of ARG1-D symptoms, if present

11.4. Physical Examination (Including Neurological Examination)

An age-appropriate physical examination will be performed by the Investigator or qualified designee during screening. The physical examination will include:

- Evaluation of the head, eyes, ears, nose, throat, cardiovascular, dermatological, musculoskeletal, respiratory, gastrointestinal, and neurological systems and recorded as normal, abnormal but not clinically significant, or abnormal and clinically significant and if the latter occurs, recorded as appropriate as an AE.

- No rectal or pelvic examinations are required.

Further details of data collection requirements will be outlined in the eCRFs. Physical examinations performed after screening (Visits 1, 4, 8, 13, and 14, Appendix 1, Table 4) will document normal, abnormal but not clinically significant, or abnormal and clinically significant physical changes from Screening. If the latter are observed, they should be recorded as AEs.

11.5. Assessment of Growth

Length, weight, and head circumference will be collected at Visits 1, 4, 8, 13, and 14 (Appendix 1, Table 4). Weight will be obtained prior to each dose with the subject in socks or bare feet and without braces, walkers, or similar appendages.

11.6. Vital Signs

Vital signs (pulse rate, blood pressure, respiration rate, and body temperature (using the same method throughout the study for a given subject) will be assessed once at each visit (Appendix 1, Table 4). The vital signs will be recorded prior to pegzilarginase administration.

11.7. Electrocardiogram

A 12-lead ECG will be obtained after approximately 5 minutes of rest in the supine position using equipment at the site at Screening and Visits 13 and 14 (Appendix 1, Table 4). The Investigator or designee will evaluate the ECGs for abnormalities and record as normal, abnormal but not clinically significant or abnormal and clinically significant. If the latter occur, they should be captured as AEs.

11.8. Efficacy Assessments: Arginine and Ornithine

Arginine will be assessed at Screening (up to 3 times as needed) and Visits 1, 2, 3, 4, 6, 8, 10, 13, and 14. Ornithine will be assessed at Screening and Visits 1 and 13 (Appendix 1, Table 4). Blood samples will be drawn pre-dose on dosing days. One additional arginine sample per subject will be collected between 24- and 48-hours post-dose at each of the Visits 2, 4, and 10 for PD analyses (Appendix 1, Table 4).

Blood samples will be sent to the central laboratory.

11.9. Efficacy Assessments: GMFM

The GMFM is a clinical measure designed to evaluate gross motor function in children with cerebral palsy by observing the subject's ability to initiate and complete certain movements. GMFM-66 consists of 66 Items. For this study, the parts of the GMFM-66 will be used as age appropriate and feasible at the Investigator's discretion for each subject for Screening and Visit 13 (Appendix 1, Table 4). GMFM-66 covers Part A through to E, using a four-point scale:

- Part A assesses lying and rolling
- Part B assesses sitting
- Part C assesses crawling and kneeling
- Part D assesses standing
- Part E assesses walking, running, and jumping.

GMFM-66 Items are ordered in terms of difficulty. Information is provided on the level of difficulty for each Item. If any individual Part A through E of the GMFM-66 assessment is not feasible and completed (missing individual items is acceptable) at Screening, that entire Part of the GMFM-66 will not be completed at Visit 13.

A four-point scale is used for the scoring of Items:

- 0 = does not initiate
- 1 = initiates
- 2 = partially completes
- 3 = completes.

11.10. Blood sampling

The EU recommends a blood draw limit of 0.8 - 0.9 mL/kg body weight (corresponding to 1% of the total blood volume) at a single time point, and 2.4 mL blood/kg body weight (corresponding to 3% of the total blood volume) within 4 weeks (EU Ethical considerations for clinical trials on medicinal products conducted with the paediatric population).

The planned blood draw volume in this study will be about 50 mL, with a blood draw volume of per visit between 1.5 mL to 8.9 mL, and within a four-week period of between 10.7 mL to 14.4 mL.

11.11. Clinical Laboratory Tests

Clinical laboratory blood samples (chemistry, haematology, coagulation) will be obtained and processed locally according to the schedule of assessments (Appendix 1, Table 4). Haematology assessments include complete blood count including RBC, white blood cell with differential count, haemoglobin, haematocrit, and platelet count. Serum chemistry assessments include sodium, potassium, chloride, blood urea nitrogen, creatinine, total bilirubin, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and lactate dehydrogenase. Coagulation tests include prothrombin time, partial prothrombin time, and international normalized ratio. Further details can be found in the study laboratory manual. Clinically significant liver enzyme elevations should be repeated and followed closely until resolution or back to baseline values. Values >3 x the upper limit of the normal range (ULN) should be reported as an AE, refer to Section 12.1.3.

11.12. Ammonia

Samples for analysis of plasma ammonia will be drawn at Screening/pre-dose Visit 1, pre-dose Visits 4 and 8, and at Visits 13 and 14 (Appendix 1, Table 4) and processed locally.

Care will be exercised in obtaining samples for ammonia testing. Institution-specific procedures for obtaining and processing samples for ammonia testing **must** be followed carefully to ensure an accurate result. A repeat test will be considered to confirm clinically significant elevations and repeated and followed closely until resolution or back to baseline values. Clinically significant elevations should be reported as an AE, refer to Section 12.1.3.

11.13. Anti-Drug Antibody Testing

Blood samples for ADA testing will be drawn at Screening/pre-dose Visit 1, pre-dose Visits 2, 4, and 8, and at Visits 13 and 14 and sent to the central laboratory (Appendix 1, Table 4). Samples will be analysed both for antibodies against pegzilarginase and against PEG.

11.14. Pharmacokinetic Assessments

Blood samples will be collected for measurement of serum concentrations of pegzilarginase pre dose at Visits 1, 2, 4, 6, 8, 10, and 13, and between 24 and 48 hours post dose at each of the Visits 2, 4, and 10. Additionally, if there are blood draws for any other reason, an extra tube should be collected, if possible, for PK analysis at the same time (if permitted by daily volume limits), and the time relative to dosing will be recorded. The timing of the required sample collection is described in Table 4. PK parameters including $T_{½}$, T_{max}, C_{max}, AUC_{0-t}, $AUC_{0-\infty}$, CL/F, and V_{ss}/F will be evaluated.

11.15. Individualised Diet Management and 3-Day Diary Diet Record

Investigators or dietitians will prescribe and attempt to maintain (within approximately ± 15 % from Baseline) the same dietary regimen as prior to enrolment throughout the study unless clinically indicated otherwise. The prescribed dietary regimen (individualised diet management questionnaire) during Screening and at Visit 13 will be collected in the eCRFs (Appendix 1, Table 4).

Caregivers will be also instructed to maintain records for all consumed dietary intake by the study subject (natural protein and medical food/EAA supplementation) for any 3 consecutive days in a diet diary issued during the Screening Period to bring to Visit 1 and issued at Visit 12 for one additional period of any 3 consecutive days after Visit 12 prior to Visit 13 to bring to Visit 13 (Appendix 1, Table 4).

Diet records will be analysed by site dietitian; total calories, grams of natural protein, and grams of synthetic protein consumed by study subject will be recorded in the eCRF. Any dietary supplements should be recorded in the concomitant medications and updated if any changes are required.

11.16. Follow-Up

All subjects will return to the study site for a follow-up assessment (Visit 13) 1 week after the last dose (Visit 12) and an SFU assessment approximately 8 weeks following the last dose of the study drug (Visit 14, Appendix 1, Table 4). See Section 10.1 for required follow-up for subjects that discontinue the study early.

11.17. Home Health Care

If appropriate, in the opinion of the Investigator, subjects may have study drug dosing, laboratory samples and/or safety assessments taken outside of the investigational site (i.e., subject's home or other appropriate location). In such cases, these protocol procedures will be performed by appropriately qualified and trained home health care personnel, under the direction of the Investigator.

12. SAFETY ASSESSMENTS AND REPORTING

Safety will be monitored during the trial with recording of AEs, routine laboratory assessments, physical examinations, growth assessments, and vital signs.

12.1. Adverse Events, Serious Adverse Events, Suspected Unexpected Serious Adverse Reactions, and Laboratory Test Abnormalities

12.1.1. Adverse Events

AE means any untoward medical occurrence in a subject to whom a medicinal product is administered, and which does not necessarily have a causal relationship with this treatment.

An AE may include intercurrent illnesses or injuries that represent an exacerbation (increase in frequency, severity, or specificity) of pre-existing conditions (e.g., worsening of asthma).

Whenever possible, it is preferable to identify and record a diagnosis as the AE term rather than a series of terms/symptoms relating to a diagnosis. Symptoms of a diagnosis should not be recorded as separate AEs (for example: do not record fever with an AE of pneumonia).

The reporting period for serious AEs (SAEs) is the period from the signing of informed consent continuing through the last study SFU visit, and for non-serious AEs from first dose of study medication through the last SFU visit. All AEs will be followed to resolution, stabilisation (maintenance of condition, presumed to remain chronic at a level other than baseline), or until return to baseline or per the Investigator's clinical judgment no further follow-up is required. Resolution is defined as the return to baseline status or recovery of the condition. If the Investigator becomes aware of an SAE with a suspected causal relationship to the medicinal product that occurs after the end of the clinical study in a subject treated by him or her, the Investigator shall, without undue delay, report the SAE to the Sponsor.

The Investigator will assess AEs for severity, causality, and seriousness.

Changes in severity or frequency: A continuous event changing in severity will be considered one event with the most severe intensity documented.

Intermittent AEs will only be recorded once, as long as the severity does not change and 'intermittent' is added to the verbatim text of the AE term. If, however, the AE changes from intermittent to continual, the original event should be closed out and reopened as a new AE.

Changes in seriousness: A continuous event with a changing seriousness will be considered as one event, but the start and stop date of the time the event is serious must be separately documented.

The severity of each AE will be assessed by the Investigator or qualified designee using the categories defined as follows:

- **Mild** – Event usually transient, requires minimal or no treatment and does not generally interfere with the participant's daily activities.
- **Moderate** – Event usually alleviated with additional specific therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort, but poses no significant or permanent risk of harm to the research participant, resulting in a low level of

inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning but poses no significant or permanent risk of harm to the participant.

- **Severe** – Event interferes with a participant's usual daily activity and may require intensive therapeutic intervention. Of note, the term "severe" does not necessarily equate to "serious".

The Investigator or their qualified designee will assess the relationship of an AE to the study treatment using the categories defined in Table 3 to determine the most likely aetiology of the event.

Table 3: Adverse Event Relationship to Study Medication

Relationship	Description
Related	There is a reasonable possibility of a causal relationship between the event and the study medication or trial procedure. This means that there are facts (evidence) or arguments to suggest a causal relationship.
Not related	There is no reasonable possibility of a causal relationship between the event and the study medication or trial procedure. This means that there are neither facts (evidence) nor arguments to suggest a causal relationship.

To classify AEs, preferred terms will be assigned by the Sponsor or contract research organization (CRO) to the original terms entered on the eCRF, using the Medical Dictionary for Regulatory Activities (MedDRA) terminology.

12.1.2. Serious Adverse Events and Suspected Unexpected Serious Adverse Reactions

An SAE is defined as any AE that:

- Results in death
- Is life threatening, that is, places the subject at immediate risk of death from the event as it occurred. This definition does not include a reaction that, had it occurred in a more severe form, might have caused death.
- Requires in-subject hospitalisation (with a minimum 24-hour stay) or prolongation of an existing in-subject hospitalisation.
- Results in persistent or significant disability or incapacity. An event qualifies as resulting in a persistent or significant disability or incapacity if it involves a substantial disruption of the subject's ability to carry out usual life functions. This definition is not intended to include experiences of relatively minor or temporary medical significance.
- Is an important medical event or serious medical condition that:
    - Does not meet any of the above criteria but
    - May jeopardize the subject or require medical or surgical intervention to prevent 1 of the outcomes listed above.

  NOTE: Examples include intensive treatment in the emergency room of allergic bronchospasm, blood dyscrasias, or convulsions that do not require hospitalisation

- More than one of the above criteria may apply to any specific AE.

The reporting period for SAEs is the period from the signing of informed consent continuing through the last study follow-up visit (8 weeks after the last dose of the study drug). SAEs must be followed by the Investigator until resolution or return to baseline or per the Investigator's clinical judgment no further follow up is required. Resolution of an SAE is defined as the return to baseline status or stabilisation of the condition, with the expectation that it will remain chronic. Preplanned or social hospitalisations should not be reported as AEs.

Although post-study events are not required to be routinely sought or collected by the Sponsor, SAEs that occur after a subject has completed the clinical study (including any protocol required post-treatment follow-up) and are considered related to study drug by the Investigator must also be reported by the Investigator to the Sponsor.

Any SAE, irrespective of whether it is considered related to study drug, will be reported in a timely manner, within 24 hours, to the Sponsor or its safety designee using the study-specific SAE form within the eCRF. Upon submission of the eCRF SAE form indicating an SAE by the Investigator, the eCRF system automatically releases a notification of the initial SAE report to the Sponsor and/or safety designee. In addition to the outcome of the SAE, any medication or other therapeutic measures used to treat the event will be recorded on the SAE form in the eCRF. Investigators will not wait to collect additional information that fully documents the event before notifying the Sponsor and/or its safety designee of an SAE. The Sponsor may be required to report certain SAEs to regulatory authorities within 7 calendar days of being notified about the event; therefore, it is important that Investigators submit additional information requested by the Sponsor, the Medical Monitor, and/or their safety designee in a timely manner once they become aware of it.

If, due to any reason, the electronic completion and forwarding of the initial SAE form via the eCRF is not possible, the Investigator must complete a paper SAE report and send it within the same timeframe to the Sponsor and/or safety designee by efax ([redacted]) or email ([redacted]), the efax or email will automatically forward the report to both parties.

The initial report should contain as much information as possible, but at least the following information:

- Subject identification
- Study drug information (date of administration)
- Event term (only one term should be entered)
- Date and time of onset

  An actual time point or best estimate (hh:dd:mm:yy) of onset of the event should be given. If the hour of onset is not available, day, month and year are acceptable. If the day of onset is not available, month and year are acceptable. If the month of onset is not available, year is acceptable.

- Name of the Investigator

- Causality assessment (relationship to IP)
- Severity.

Additional follow-up information, if required or available, must be transmitted to the Sponsor and/or safety designee within 24 hours of receipt. This follow-up information will be completed on a follow-up SAE form within the eCRF and submitted to the Sponsor and/or safety designee as described above for the initial SAE form.

The Investigator is encouraged to discuss with the Medical Monitor any AEs for which the issue of seriousness is unclear or questioned. Contact information for the Medical Monitor is provided in the Investigator site file regulatory binder provided to each site.

A serious unexpected serious adverse reaction (SUSAR) is a suspected adverse reaction related to the study medication that is both unexpected and serious. SUSARs will be subject to expedited regulatory reporting.

SUSARs will be expedited to independent ethics committees, competent authority, and Investigator following pertinent national legislation.

Within the EU all relevant information about any SUSAR will be reported electronically and without delay by the Sponsor to the EudraVigilance database.

The reporting period for SUSARs is based on the severity of the reaction and is determined as follows:

- fatal or life-threatening SUSARs, as soon as possible and in any event not later than 7 days after the Sponsor became aware of the reaction
- non-fatal or non-life-threatening SUSARs, not later than 15 days after the Sponsor became aware of the reaction
- SUSARs which were initially considered to be non-fatal or non-life-threatening, but which turn out to be fatal or life-threatening, as soon as possible and in any event not later than 7 days after the Sponsor became aware of the reaction being fatal or life-threatening.

12.1.3. Laboratory Test Abnormalities

The Investigator will routinely review all safety laboratory results. Out-of-range laboratory test values that the Investigator considers to be a clinically significant change from the subject's baseline value or previous values will be reported on the AE page of the eCRF. Clinically significant abnormal laboratory test results should be reported as AEs and will be repeated and followed until a return to normal or baseline values or per the Investigator's clinical judgment no further follow up is required.

12.2. Hypersensitivity Reactions, Injection Site Reactions, Hyperammonaemic Episodes, and Prolonged Hypoargininaemia

HSRs (Section 12.2.1), ISRs (Section 12.2.2), hyperammonaemic episodes (Section 12.2.3), and prolonged hypoargininaemia (Section 12.2.4) will be closely monitored in this clinical study.

HSRs and ISRs are associated with biologic therapy and have been observed with pegzilarginase administration.

12.2.1. Hypersensitivity Reactions

If, at any time during the study, the Investigator observes symptoms that he/she/they considers to be consistent with an HSR related to administration of study drug, the symptoms will be recorded as an AE(s) and designated as an HSR. Examples of symptoms of potential HSRs after dosing include but are not limited to a constellation of signs and symptoms of flushing, fever and/or chills, pruritus, urticaria, facial and/or tongue oedema, chest pain, dyspnoea, wheezing, stridor, hypotension or hypertension, bradycardia, or tachycardia. HSRs should be managed according to standard of care.

Any subject who experiences an HSR must safely receive at least 4 subsequent doses thereafter at the study site, after which dosing by home health care may be considered if deemed safe by the Investigator and after consultation with the Sponsor.

HSRs observed in pegzilarginase studies were typically managed with antihistamines. There were no subjects that withdrew from the previous clinical studies due to an HSR. Additional information is available in the SmPC.

General guidelines for classifying the severity of a reaction are provided below:

- Mild reactions are defined as self-limiting, spontaneously resolved reactions and may be managed with a temporary interruption of weekly dosing
- Moderate reactions are defined as reactions that do not resolve with simple measures; require extended observation and therapy interruption
- Severe reactions are defined as reactions that require intervention to prevent a serious outcome.

12.2.2. Injection Site Reactions

ISRs are common effects of biologic treatments given via SC injection. They are a local phenomenon defined as a constellation of symptoms, including swelling, erythema, pruritus, and pain around the site of the injection. ISRs should be managed according to standard of care.

If, at any time during the study, the Investigator observes symptoms that he/she/they considers to be consistent with an ISR related to administration of study drug, the symptoms will be recorded as an AE(s) and designated as an ISR.

12.2.3. Hyperammonaemic Episodes

HA occurs in some patients with ARG1-D as part of the disease. Therefore, plasma ammonia will be monitored throughout the study treatment period (Section 11.12 and Appendix 1, Table 4). The effects of HA are not always symptomatic.

Cases of increased ammonia without symptoms or not needing hospitalisation or emergency room care will be recorded as an AE of "elevated ammonia."

Cases of "hyperammonaemic episodes" will be defined as events that meet all 3 of the following:

- HA with a confirmed ammonia level ≥ 100 µM
- Symptoms related to HA
- Requiring hospitalisation or emergency room management, with or without admission to the hospital.

Cases of hyperammonaemic episodes should be recorded as an AE of "hyperammonaemia". Upon the occurrence of any hyperammonaemic episode, repeat plasma ammonia samples will be taken to confirm the plasma ammonia level and document resolution (local laboratory). Hyperammonaemic episodes should be managed according to standard of care.

12.2.4. Prolonged Hypoargininaemia

Sustained plasma arginine levels below the lower limit of normal is a consideration for patients treated with pegzilarginase. In the study, plasma arginine levels will be monitored frequently, with the longest period between sampling of 14 days, and hence a risk of prolonged hypoargininaemia is limited.

Cases of "prolonged hypoargininaemia" will be defined as events of continuous plasma arginine levels below the lower limit of the normal (LLN) for 14 days or longer.

Cases of prolonged hypoargininaemia should be recorded as an AE of "prolonged hypoargininaemia". Upon the occurrence of any sustained hypoargininaemia, repeat plasma arginine samples should be taken to confirm the plasma arginine level and document resolution.

12.3. Treatment of Overdose

There is no experience of overdose with pegzilarginase in ARG1-D. Potential effects from an overdose would likely be as a result of an exaggerated pharmacologic effect of pegzilarginase resulting in abnormally low plasma arginine levels.

In a Phase 1 study in subjects with advanced solid tumours, 1 subject inadvertently received 1.6 mg/kg of pegzilarginase (16 × the recommended starting dose of 0.1 mg/kg in ARG1-D patients). The subject developed nausea, vomiting, diarrhoea, and fatigue and was successfully treated with intravenous supportive care without sequelae.

Subjects suspected of receiving an overdose should be closely monitored and general supportive measures should be initiated as there is no specific antidote for pegzilarginase overdose.

12.4. Medication error, overdose, abuse, and misuse

Medication errors, overdose, abuse, and misuse must be documented on the respective eCRF page and are defined as follows:

- Medication error: Any unintentional error in the prescribing, dispensing or administration of the study medication while in the control of the healthcare professional, subject, or consumer. Examples for medication errors include administration of expired study medication, administration of study medication that has undergone temperature excursion from the specified storage range, use of study medication outside of what is foreseen in the protocol, administration of study medication to subjects not involved in the study or foreseen to receive another study medication.
- Overdose: Administration of a quantity of the study medication given per administration or cumulatively which is above the maximum dose.
- Abuse: Persistent or sporadic, intentional excessive use of the study medication which is accompanied by desired physical or psychological effects.
- Misuse: Situation where the study medication is intentionally and inappropriately used not in accordance with the protocol.

Stop and start date as well as the action with study medication due to medication errors, overdose, abuse, or misuse must be documented as follows:

- Drug withdrawn
- Dose reduced
- Dose increased
- Dose not changed
- Unknown
- Not applicable.

If a medication error, overdose, abuse, and misuse is accompanied by an AE, as determined by the investigator, the AE should be recorded on the AE page of the eCRF.

12.5. Annual Safety Reporting

In line with the provisions of pertinent national legislation, the Clinical Trials Regulation (EU) No 536/2014, where applicable, and the International Council for Harmonisation (ICH) Guideline E2F, the Sponsor will submit an annual report on the safety of the study medication.

12.6. Independent Safety Review Committee and Safety Reviews

A formal Safety Review Committee is not planned for this study. Safety will be monitored by the Sponsor and the study Investigators.

13. STATISTICAL CONSIDERATIONS

13.1. Sample Size Determination

The sample size of 3 subjects is solely based on clinical considerations and the EU Paediatric Investigational Plan.

13.2. Populations for Analyses/Analysis Sets

All analyses will be performed on the Full Analysis Set, defined as all subjects who are enrolled and receive at least 1 dose of study drug. Subjects are considered to be enrolled at the time of signing the consent form.

13.3. Statistical Analyses

Endpoints and other assessments will be presented using descriptive statistics only. Continuous variables will be presented using the n, mean, standard deviation, median, minimum, and maximum. Categorical variables will be presented using number and percentage per category. All analyses will be fully described in the statistical analysis plan (SAP) for each study endpoint. Deviations from the analyses planned in the protocol will be addressed in the SAP and/or study report as appropriate.

Formal hypothesis testing will not be performed.

Appropriate subject listings will be provided for efficacy and safety data.

13.3.1. Disposition and Demographics

Subject disposition, demographics, baseline characteristics, medical history, prior and concomitant medications will be summarised using applicable descriptive statistics.

13.3.2. Analysis of Primary Endpoint

Primary endpoint is change from Baseline in plasma arginine after 12 weeks of pegzilarginase treatment. Absolute values, absolute change and percentage change will be tabulated for plasma arginine by study visit.

13.3.3. Analysis of Secondary Endpoints

Safety Assessments

Adverse events will be summarised by MedDRA system organ class (SOC) and preferred term including HSRs, ISRs, hyperammonaemic episodes and prolonged hypoargininaemia.

A TEAE is defined as an AE that begins or worsens (increases in severity) on or after the date (and time if known) of the first dose of the study drug. The number and percentage of subjects with TEAEs, related TEAEs, serious TEAEs, related serious TEAEs, discontinuation due to an AE, dose interruptions due to an AE, and fatal AEs will be summarised by MedDRA SOC and preferred term.

Summaries of AEs by maximum severity will be produced.

Summaries of concomitant medications will be produced.

Laboratory data (chemistry, haematology, coagulation) and plasma ammonia, vital signs and ECG data will be summarised including changes from Baseline. Results of the physical examination will also be tabulated.

Longitudinal changes from Baseline in anthropometric data will be assessed for length-for-age, weight-for-age, weight-for-length, and head circumference-for-age determined from the publicly available World Health Organization (WHO) growth curves (for subjects < 24 months of age) and Centers for Disease Control and Prevention (CDC) growth curves (for subjects who age >24 months during the course of the study) and summarised (CDC, Growth Charts).

PK Parameters

Details of the PK analysis will be in a separate analysis plan.

PD Response Evaluation

ADAs and levels of plasma arginine and ornithine will be summarized. Proportion of subjects within the normal range and within the guideline level (<200 μM) will be presented.

Changes in physical function after 12 weeks of pegzilarginase

GMFM-66 Parts A through E scores, and their corresponding change from Baseline, and percent change from Baseline will be summarised using applicable descriptive statistics by study visit.

13.4. Interim Analyses

No interim analysis is planned for this study.

13.5. Handling of missing, unused, and spurious data

Statistical analyses will be done using observed values only. No imputation of missing values (e.g., last observation carried forward) is planned, unless stated otherwise.

13.6. Analysis of study conduct and subject disposition

The disposition of subjects will be shown. Inclusion and exclusion criteria, and status at the end of the study will be presented. In case of premature discontinuation, reasons for discontinuation will be described.

14. SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

14.1. Regulatory and Ethical Considerations

This study will be conducted in accordance with the protocol and with the following:

- Applicable ICH Good Clinical Practice (GCP) Guidelines
- Clinical Trials Regulation (EU) No 536/2014, if applicable
- Declaration of Helsinki in its currently acknowledged version
- Applicable laws and regulations.

The protocol, protocol amendments, if applicable, subject information sheet and ICF, and other relevant documents (e.g., advertisements) must be approved by the competent Independent Ethics Committee(s) (IEC) and/or authorised by the competent authority, as applicable, before the study is initiated.

Substantial modifications to the trial documents must be approved and/or authorised before implementation, except for changes necessary to eliminate an immediate hazard to study subjects.

If applicable, any additional requirements imposed by the IEC, e.g. periodic reporting, will be followed.

14.2. Privacy and Confidentiality

The confidentiality of subject records will be protected in accordance with applicable laws, regulations, and guidelines in all regions where the study is conducted.

After subjects have consented to take part in the study, the Sponsor and/or its representatives may review their medical records and data collected during the study. These records and data may, in addition, be reviewed by others including the following: independent auditors who validate the data on behalf of the Sponsor; third parties with whom the Sponsor may develop, register, or market pegzilarginase; national or local regulatory authorities. The Sponsor and/or its representatives accessing the records and data will take all reasonable precautions in accordance with applicable laws, regulations, and guidelines to maintain the confidentiality of subjects' identities.

Subjects are assigned a unique identifying number; however, their initials and date of birth (or age where date of birth may not be collected) may also be collected and used to assist the Sponsor to verify the accuracy of the data (e.g., to confirm that laboratory results have been assigned to the correct subject).

The results of studies containing subjects' unique identifying number, relevant medical records, and possibly initials and dates of birth, will be recorded. They may be transferred to, and used in, other countries which may not afford the same level of protection that applies within the countries where this study is conducted. The purpose of any such transfer would include supporting regulatory submissions, conducting new data analyses to publish or present the study results, or answering questions asked by regulatory or health authorities.

In case of a data security breach, potential adverse effects will be mitigated as per the mechanisms which the Sponsor as the data controller has implemented based on the obligations set out by applicable regulations, e.g., Regulation (EU) 2016/679 (General Data Protection Regulation).

14.3. Data Quality Assurance

All subject data relating to the study will be recorded on the eCRF unless transmitted to the Sponsor or designee electronically (e.g., laboratory data). The Investigator is responsible for verifying that data entries are accurate and correct by physically or electronically signing the eCRF.

The Investigator must maintain accurate documentation (source data) that supports the information entered in the eCRF.

The Investigator and institution must permit study-related monitoring, audits, and regulatory agency inspections, and must provide direct access to source data documents.

14.4. Management of Deviations

A protocol deviation is a failure to follow, intentionally or unintentionally, the requirements of the protocol.

In emergency circumstances, deviations from the protocol may proceed without prior consultation with the Sponsor and favourable opinion of the IEC, if the rights, safety, and well-being of human trial participants need to be protected. Such deviations will be documented and reported to the Sponsor and the IEC as soon as possible in accordance with national regulations.

All protocol deviations will be listed. For the reporting of a serious deviation refer to Section 14.5.

14.5. Serious Breaches

Concerning the definition of a serious breach, the applicable legislation must be adhered to and guidance relevant to the concerned countries must be consulted. If a serious breach is suspected, the Sponsor and Sponsor's designee must be contacted within 1 working day by telephone or email. Details of the relevant person(s) to be contacted can be found in the contact list provided in the Investigator site file. In collaboration with the Investigator and Sponsor's designee, the serious breach will be reviewed by the Sponsor and, if appropriate, the Sponsor or Sponsor's designee will notify the regulatory authorities and IECs, as applicable, without undue delay but not later than 7 calendar days of becoming aware of that breach.

14.6. Case Report Forms and Source Documents

Subject data from source documents will be entered directly into the eCRFs at the Investigator sites. No data will be recorded directly in the eCRF without records in source data documents. Definitions on what constitutes source data can be found in the monitoring plan. The Investigator is responsible for assuring that source documentation is appropriately maintained, and that data entered into the eCRF is complete and accurate, and that entries and updates are performed in a timely manner.

The Sponsor or designees will review the data entered by investigational staff for completeness and accuracy. Data queries stating the nature of the problem and requesting clarification will be created for discrepancies and missing values and sent to the investigational site. Designated Investigator site staff are required to respond promptly to queries and to make any necessary changes to the data.

14.7. Study and Site Closure

The Sponsor reserves the right to terminate the study, and the Investigator reserves the right to terminate their involvement in the study, according to the study contract at any time.

14.8. Use of Information and Publication Policy

The study will be registered before the inclusion of the first subject in a public register according to local regulations.

After completion of the study, the results will be summarized in a clinical study report according to the ICH E3 Note for guidance on structure and content of clinical study reports.

Within 6 months of the end of the study, the Sponsor shall submit a summary of the results of the clinical study and, where appropriate, a summary in a form understandable to lay persons, in accordance with the relevant national provisions and, where applicable, the Clinical Trials Regulation (EU) No 536/2014.

The Sponsor recognizes the importance of communicating medical study data and therefore encourages publication in reputable scientific journals and at seminars or conferences. The details of the processes of producing and reviewing reports, manuscripts, and presentations based on the data from this study will be described in the clinical study agreement between the Sponsor, CROs, and the Investigator.

Due to the confidential nature of this development program, the results of the study may not be published or publicly presented without the prior approval of the Sponsor. Any Investigator wishing to publish or present any study finding must present a manuscript or abstract to the Sponsor 120 days prior to submission for publication or presentation to provide the Sponsor an opportunity for review and comment.

14.9. Retention of Records

The Investigator must retain all study records required by the Sponsor and by the applicable regulations in a secure and safe facility. The Investigator must consult a Sponsor representative before disposal of any study records and must notify the Sponsor of any change in the location, disposition, or custody of the study files. The Investigator/institution must take measures to prevent accidental or premature destruction of essential documents (i.e., documents that individually and collectively permit evaluation of the conduct of a study and the quality of the data produced), including paper copies of study records (e.g., subject charts), as well as any original source documents that are electronic as required by applicable regulatory requirements.

All essential documents at the study centre, must be retained by the Sponsor or Sponsor’s designee for at least 2 years after the last approval of a marketing application in the US or an ICH region, until there are no pending or contemplated marketing applications in the US or an

ICH region, until ≥2 years have elapsed since the formal discontinuation of clinical development of the investigational medicinal product, and at least 25 years after the end of trial, whichever period is longer. The final report will be kept for another 5 years after the investigational product has been taken from the market according to the legal stipulations. The documents should be archived for a longer period, if required by the applicable regulatory authorities or if agreed with the Sponsor. The Sponsor is responsible to inform the Investigators when these documents have to be retained no longer. The medical files of trial subjects must be retained in accordance with local legislation and in accordance with the maximum period of time permitted by the hospital, institution, or private practice.

14.9.1. Study Monitoring, Auditing, and Inspections

Qualified individuals designated by the Sponsor will monitor all aspects of the study according to ICH/GCP and standard operating procedures for compliance with applicable regulations. The Investigator agrees to allow these monitors direct access to the clinical supplies, dispensing, and storage areas and to the clinical files, including original medical records of the study subjects, and, if requested, agrees to assist the monitors. The Investigator and staff are responsible for being present or available for consultation during routinely scheduled site visits conducted by the Sponsor or its designees.

The review of the subjects' medical records will be performed in a manner to ensure that subject confidentiality is adequately maintained. Further details of the study monitoring will be outlined in a Monitoring Plan.

Members of the Sponsor's Quality Assurance Department or designees may conduct an audit of a clinical site or any vendor at any time during or after completion of the study. The Investigator or vendor will be informed if an audit is to take place and advised as to the scope of the audit.

Representatives of regulatory agencies may also conduct an inspection of the study site and corresponding documentation. If informed of such an inspection, the Investigator will notify the Sponsor immediately. The Investigator will ensure that the auditors have access to the clinical supplies, study site facilities, original source documentation, and all study files. All inspections and audits will be carried out giving consideration to data protection as well as subject confidentiality to the extent that local, state, and federal laws apply.

If planned on-site monitoring visits are not possible, use of central and remote monitoring programs should be optimized to maintain oversight of clinical sites. In line with local laws and regulations remote source data verification may be part of the remote monitoring visits and if so, must be agreed between the Sponsor's designee and the study centre. If necessary, the relevant study documents (e.g., monitoring plan) will be adjusted to reflect these activities.

The ICH-GCP requirements and applicable data protection and privacy regulations must be met in any case and for any selected monitoring approach. In case of a remote monitoring of data, the participants need to agree to it in the ICF.

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APPENDIX 1 SCHEDULE OF ASSESSMENTS

Table 4 Schedule of Assessments

Study Period	Screening	Treatment												Follow-Up	
Visit ID	**Screening**	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**	**V7**	**V8**	**V9**	**V10**	**V11**	**V12**	**V13 or ET[h]**	**V14/SFU**
Study Day	**-28 to -1**	**1**	**8**	**15**	**22**	**29**	**36**	**43**	**50**	**57**	**64**	**71**	**78**	**85**	**134**
Window	**≤4 weeks**	**±1d**	**±1d**	**±1d**	**±1d**	**±1d**	**±1d**	**±1d**	**±1d**	**±1d**	**±1d**	**±1d**	**±1d**	**±1d**	**±7d**
Study Week	**NA**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**20**
Informed Consent/Subject															
Informed Consent consent	X														
Inclusion/ Exclusion Criteria	X														
Demographics	X														
Disease Characteristics	X														
Medical and Surgical History and Prior Medications	X														
Confirm diagnosis of ARG1-D[a]	X														
Clinical Assessments															
Length, Weight, Head Circumference		X			X				X					X	X
Vital Signs[b]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
ECG	X													X	X
Physical Examination	X	X			X				X					X	X
Laboratory Assessments[c]															
Arginine[b]	X[e]	X	X	X	X		X		X		X			X	X
Ornithine[b]	X	X												X	
PK[d]		X	X		X		X		X		X			X	

Study Period	Screening	Treatment												Follow-Up	
Visit ID	**Screening**	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**	**V7**	**V8**	**V9**	**V10**	**V11**	**V12**	**V13 or ET[h]**	**V14/SFU**
Study Day	**-28 to -1**	**1**	**8**	**15**	**22**	**29**	**36**	**43**	**50**	**57**	**64**	**71**	**78**	**85**	**134**
Window	**≤4 weeks**	**±1d**	**±1d**	**±1d**	**±1d**	**±1d**	**±1d**	**±1d**	**±1d**	**±1d**	**±1d**	**±1d**	**±1d**	**±1d**	**±7d**
Study Week	**NA**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**20**
PK and Arginine (24-48 hr post-dose)[f]			X		X						X				
ADA[b]	X[g]		X		X				X					X	X
Haematology[b]	X[g]				X				X					X	X
Chemistry[b]	X[g]				X				X					X	X
PT/PTT/INR[b]	X[g]													X	X
Ammonia[b]	X[g]				X				X					X	X
Neuromotor Assessment															
GMFM-66 Parts A to E, as age appropriate and feasible	X													X	
Other															
Individualised Diet Management Questionnaire	X													X	
Issue Subject Diet Diary	X												X		
Collect Subject Diet Diary		X												X	
Drug Administration															
Pegzilarginase SC Injection		X	X	X	X	X	X	X	X	X	X	X	X		
Safety															
Adverse Events	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant Medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

ADA = anti-drug antibody; ARG1-D = arginase 1 deficiency; ECG = electrocardiogram; ET = early termination; GMFM = Gross Motor Function Measure; ID = identification; INR = international normalized ratio; NA = not applicable; PD = pharmacodynamic; PK = pharmacokinetic; PT = prothrombin time; PTT = partial thromboplastin time; RBC = red blood cell; SC = subcutaneous; SFU = safety follow-up; V = Visit

Notes:

[a]: Diagnosis by elevated plasma arginine levels, a mutation analysis that results in a pathogenic variant, or RBC arginase activity.

[b]: Pre-Dose on dosing days.

[c]: Additional assessments to be done per clinical judgement.

[d]: PK samples will be collected pre-dose within 1 hour on dosing days. In case of blood draws for other reasons, an extra tube should be collected, if possible, for PK analysis at the same time (if permitted by total daily volume limits), and the time relative to dosing will be recorded.

[e:] Arginine can be assessed up to 3 times during the screening period. The highest value will be used for Baseline.

[f]: An additional PK and arginine sample will be collected anytime between 24-48 hours post-dosing after Visits 2, 4, and 10.

[g]: Assessments to be done anytime within the 6 days prior to dosing or on the day of dosing. If assessments are done more than 6 days prior to dosing, they must be repeated within the 6 days prior to dosing.

[h:] If a subject discontinues treatment early from the study, V13 and V14 should be conducted per the schedule of assessments to assess efficacy and safety.