1. TITLE PAGE

Protocol Title: An Open-Label, Multicenter Study to Evaluate the Long-Term Safety, Tolerability, and Efficacy of AEB1102 in Patients with Arginase 1 Deficiency

Protocol Number:	CAEB1102-102A
Amendment Number:	Version 6.0
Compound Name/Number:	Pegzilarginase/AEB1102
Study Phase:	Phase 2
Sponsor Name:	Aeglea BioTherapeutics, Inc.
Local Registered Address:	805 Las Cimas Pkwy Suite 100, Building III Austin, TX 78746
IND Number	127774
EudraCT Number:	2018-003163-67
Protocol History:	Amendment 6.0: May 21, 2021 Amendment 5.0: October 9, 2020 Amendment 4.0: May 5, 2020 Amendment 3.0: October 17, 2019 Amendment 2.1: May 8, 2019 (UK country specific) Amendment 2.0: March 28, 2019 Amendment 1.2: November 30, 2018 (UK country specific) Amendment 1.1: May 14, 2018 (Withdrawn) Amendment 1: April 17, 2018 Original Protocol: March 09, 2017

SIGNATURE PAGE

Sponsor's Approval

The protocol has been electronically approved by Aeglea BioTherapeutics, Inc in June 2021.

Responsible Medical Officer: [redacted]

Sponsor's Authorized Officer:

[redacted]

Aeglea BioTherapeutics, Inc.
805 Las Cimas Pkwy
Suite 100, Building III
Austin, TX 78746
United States
Tel: 512-942-2935

INVESTIGATOR'S AGREEMENT

I have read the Study CAEB1102-102A Amendment 6.0 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 Investigator

_____

Signature of Investigator

_____

Date

2. SYNOPSIS

NAME OF COMPANY Aeglea BioTherapeutics, Inc. 805 Las Cimas Pkwy Suite 100, Building III Austin, TX 78746 **NAME OF FINISHED PRODUCT:** Pegzilarginase (AEB1102)	**SUMMARY TABLE** Referring to Part of the Dossier: Volume: Page: Reference:	**FOR NATIONAL AUTHORITY USE ONLY:**
TITLE OF STUDY: An Open-Label, Multicenter Study to Evaluate the Long-Term Safety, Tolerability, and Efficacy of AEB1102 in Patients with Arginase 1 Deficiency		
PROTOCOL NUMBER: CAEB1102-102A		
STUDY SITES: Up to 15 sites in the US, Canada, and Europe		
STUDY PERIOD: Until pegzilarginase is available by other means	**PHASE OF DEVELOPMENT:** Phase 2	

OBJECTIVES:

The primary objective of the study is:

- To evaluate the long-term safety and tolerability of intravenous (IV) or subcutaneous (SC) pegzilarginase administered in patients with Arginase 1 deficiency and hyperargininemia

The secondary objectives of the study are:

- To evaluate the long-term effects of pegzilarginase in patients with Arginase 1 deficiency and hyperargininemia on sustained reduction of arginine levels
- To evaluate the long-term effects of pegzilarginase on plasma guanidino compounds
- To characterize repeat-dose pharmacokinetics (PK) of pegzilarginase administered IV or SC
- To evaluate the long-term effects of pegzilarginase on stabilization or improvement of neurological/neuromotor manifestations, using objective measures:
  - Six-Minute Walk Test (6MWT)
  - Berg Balance Scale (BBS)
  - Gross Motor Function Measure (GMFM)
  - Modified Ashworth Scale (MAS)
  - Purdue Pegboard Test
- To evaluate the long-term effects of pegzilarginase on select manifestations of Arginase 1 deficiency:
  - Frequency of seizures
  - Frequency of hyperammonemic episodes
- Incidence of disease-related transaminase elevations

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Exploratory Objectives:

- To compare the PK and pharmacodynamics (PD) of different routes (IV and SC) of pegzilarginase administration
- To examine the long-term changes in exploratory biomarkers including additional amino acids (ornithine, glutamine, leucine, isoleucine, and valine) and blood urea nitrogen after administration of pegzilarginase
- To evaluate the long-term effects of pegzilarginase on stabilization or improvement of neurocognitive, developmental, and quality of life (QoL) measures, selected based on patient age:
  - Intelligence:
    - Wechsler Adult Intelligence Scale IV (WAIS-IV)
    - Wechsler Intelligence Scale for Children V (WISC-V)
    - Wechsler Preschool and Primary Scale of Intelligence IV (WPPSI-IV)
    - Bayley Scales of Infant Development III (BSID-III)
  - Memory:
    - Rey Auditory Verbal Learning Test (RAVLT)
    - Atlantis subtest on the Kaufman Assessment Battery for Children II (KABC-II)
  - Attention:
    - Test of Variables of Attention (TOVA) version 9
  - Adaptive Behavior:
    - Vineland Adaptive Behavior Scales II (VABS-II)
  - Development:
    - Growth parameters (length/stature, weight, head circumference)
  - Quality of Life:
    - Patient-Reported Outcomes Measurement Information System (PROMIS) or Pediatric Quality of Life Inventory (PedsQL)
    - Short Form Zarit Burden Interview (ZBI-12)

METHODOLOGY:

This study is designed to evaluate the safety, tolerability, and efficacy of long-term IV and SC administration of pegzilarginase for the treatment of pediatric and adult patients with Arginase 1 deficiency and hyperargininemia. This study will provide long-term, open-label treatment for patients completing Study CAEB1102-101A (hereafter referred to as Study 101A) who are eligible for continued treatment with pegzilarginase.

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Study Design

This is a long-term, open-label extension study of pegzilarginase in approximately 10 pediatric and adult patients with Arginase 1 deficiency and hyperargininemia who participated in Study 101A. The Investigator and the Sponsor will evaluate each individual patient's safety information available at the time of Study 101A completion to determine if it is acceptable for the patient to be enrolled in this study. Patients must have tolerated the single and repeat-doses of pegzilarginase without experiencing any clinically significant event that would preclude continued dosing and must continue to meet applicable eligibility criteria from the original study.

The pegzilarginase dose level and frequency will initially match the dose and regimen the patient was receiving at the end of Study 101A but may be adjusted if clinically indicated in the opinion of the Investigator in consultation with the Sponsor. During the course of the study, doses, regimens, and route of administration may be adjusted for an individual based on available PK/PD data or for safety and/or tolerability reasons as clinically indicated in the opinion of the Investigator in consultation with the Sponsor. Changes to route of administration should be avoided during the first 24 weeks of the study.

The first 24 doses in this study will be administered using the IV route weekly. The first 12 IV doses will be administered in the clinical research unit [CRU]. After the initial 12 IV doses, patients may be dosed outside of the CRU (ie, by home health care professionals) if considered safe and appropriate in the opinion of the Investigator in consultation with the Sponsor.

The recommended dosing schedule is as follows:

- IV administration once weekly (QW) for Weeks 1 to 24
- SC administration QW for Weeks 25 to 48
- As clinically indicated thereafter

Safety, tolerability, immunogenicity, PK, and PD assessments as evaluated in Study 101A will continue for the duration of this long-term extension study, although with different frequency. The Data Safety Monitoring Board (DSMB) will periodically provide independent review of the safety, tolerability, immunogenicity, PK, and PD measures during the study.

A follow-up visit will be scheduled approximately 2 weeks after the completion of the final dose of pegzilarginase.

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NUMBER OF SUBJECTS PLANNED: All qualified pediatric and adult patients who participated in the 101A study will be allowed to participate in this study.		

DIAGNOSIS AND ALL CRITERIA FOR INCLUSION AND EXCLUSION:
INCLUSION CRITERIA:

A patient must meet all of the following criteria to be enrolled in this study:

1. Completed participation in Study CAEB1102-101A without experiencing any clinically significant adverse event (AE) or other unmanageable drug toxicity that precludes continued dosing
2. After review of the patient's data from the CAEB1102-101A study, the Investigator and the Sponsor confirm that it is acceptable for the patient to continue dosing with pegzilarginase and that the patient continues to meet the following criteria from the 101A study:
   - Is male or female: pediatric patients ≥2 to <18 years of age and adult patients ≥18 years of age
   - Has documented diagnosis of Arginase 1 deficiency, with:
     - Known mutation in the Arginase 1 gene

     OR

     - Known deficiency in red blood cells (RBCs) arginase activity
   - Has adequate organ function defined as follows:
     - Bone Marrow: Hemoglobin ≥10 g/dL; absolute neutrophil count ≥ 1.5 x10^9/L; platelet count ≥100,000/µL
     - Hepatic: transaminase levels (aspartate aminotransferase/alanine aminotransferase ≤2.5-times upper limit of normal (ULN); total bilirubin ≤2.0 mg/dL
     - Renal: serum creatinine <1.5 x ULN
3. If female and of child-bearing potential, has a negative serum pregnancy test within 7 days before enrollment
4. If the patient (male or female) is engaging in sexual activity that could lead to pregnancy, must be surgically sterile, post-menopausal (female), or must agree to use a highly effective method of birth control during the study and for a minimum of 30 days after the last study drug administration. Highly effective methods of contraception include: combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation; progesterone-only hormonal contraception associated with inhibition of ovulation; intrauterine device (IUD); or intrauterine hormone-releasing system (IUS).
5. Patient or legal guardian is able and willing to provide written informed consent and where required assent, and to comply with all requirements of study participation (including all study procedures and continuation of prescribed diet without modification), prior to any screening procedures

EXCLUSION CRITERIA:

1. Clinically significant concurrent disease, serious intercurrent illness, or other extenuating circumstances that, in the opinion of the Investigator, would preclude the patient's participation in the study including:
   a. Had transfusion of ≥2 units of RBCs within the 60 days before enrollment

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b. Currently has an active infection requiring systemic treatment
c. Known infection with human immunodeficiency virus (HIV), hepatitis B, or hepatitis C
d. Currently has uncontrolled hyperammonemia (confirmed ammonia ≥100 μmol/L)
e. Is currently participating in another therapeutic clinical trial
f. Has received any investigational agent (other than pegzilarginase) within 30 days of enrollment
g. If female, is lactating or breast feeding

DOSE, ROUTE AND REGIMEN:

Dosing in an individual patient will commence approximately 4 weeks (but no sooner than 3 weeks) after the last dose of study drug in Study 101A. The pegzilarginase dose level and frequency will initially match the dose and regimen the patient was receiving at the end of Study 101A but may be adjusted if clinically indicated in the opinion of the Investigator in consultation with the Sponsor.

During the course of the study, doses and route of administration may be adjusted for an individual based on available PK/PD data or for safety and/or tolerability reasons. Dose adjustments may be made to find a dose and regimen that maintains plasma arginine below the target guidance value of 200 μM, and if achievable in the normal range (40 and 115 μM) for as much time as possible during each weekly dosing period. Patients may not receive doses above the maximum tolerated dose (MTD) determined in the oncology trials of 0.33 mg/kg. Changes to route of administration should be avoided during the first 24 weeks of the study.

The first 24 doses in this study will be administered IV. The first 12 doses in this study should be administered IV weekly in the CRU. After the initial 12 IV doses, patients may be dosed outside of the CRU (ie, by home health care professionals) if considered safe and appropriate in the opinion of the Investigator in consultation with the Sponsor.

The recommended dosing schedule is as follows:

- IV administration QW for Weeks 1 to 24
- SC administration weekly for Weeks 25 to 48
- As clinically indicated thereafter (Investigator decision in consultation with the Sponsor)

DURATION OF TREATMENT:

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Each patient is expected to receive pegzilarginase following the completion of Study 101A until it becomes available via other means.

REFERENCE THERAPY, DOSE, ROUTE AND REGIMEN:

Not applicable.

OUTCOME MEASURES:

Criteria for Evaluation

Primary Endpoints:

Safety and tolerability will be assessed by:

Adverse events (AEs)/serious AEs (SAEs)

- Physical examinations
- Vital signs
- Electrocardiograms (ECGs)
- Clinical laboratory studies (serum chemistries, hematology, coagulation, urinalysis)
- Low plasma arginine levels associated with clinically significant symptoms
- Clinically significant hyperammonemia
- Immunogenicity safety measures (levels of anti-drug antibodies [ADAs], anti-polyethylene glycol [PEG] antibodies)

Secondary Endpoints:

- Changes in plasma arginine levels
- Changes in plasma guanidino compound levels
- PK parameters
- Neurological/neuromotor manifestations, using functional and spasticity measurements:
  - 6MWT
  - BBS
  - GMFM
  - MAS
  - Purdue Pegboard Test
- Other manifestations of Arginase 1 deficiency
  - Seizure frequency
  - Frequency of hyperammonemic episodes, as measured from laboratory evaluations of ammonia levels and relevant AEs
  - Incidence of transaminase elevations, as measured from laboratory evaluations of liver function tests and relevant AEs

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Exploratory Endpoints:

- Comparison of the PK and PD of pegzilarginase between different routes of administration (IV and SC)
- Changes in exploratory biomarkers after long-term administration of pegzilarginase:
  - Additional amino acids (ornithine, glutamine, leucine, isoleucine, and valine)
  - Blood urea nitrogen (BUN)
- The long-term effects of pegzilarginase on neurocognitive, developmental, and QoL, will be measured for the following categories:
  - Intelligence (WAIS-IV, WISC-V, WPPSI-IV, BSID-III)
  - Memory (RAVLT, KABC-II)
  - Attention (Test of Variables of Attention version 9)
  - Adaptive Behavior (VABS-II)
  - Development (growth parameters of length/stature, weight, head circumference)
  - Quality of Life (PROMIS or PedsQL or Short Form Zarit Burden Interview [ZBI-12])

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STATISTICAL METHODS:

General Considerations

A statistical analysis plan (SAP) will provide a detailed description of the planned statistical analyses. The SAP will be finalized prior to database lock. Any changes to the methods set forth in the SAP will be described in the final clinical study report.

Most analyses will be descriptive, presenting the data using summary statistics and/or graphs. Statistical tests may be performed as part of the descriptive analyses, as a measure of observed effects of pegzilarginase relative to observed variability. Such tests will be performed at 2-sided $\alpha = 0.05$ unless otherwise stated and will not be adjusted for multiplicity. The term "statistically significant" may be used to describe results where the p-value is no greater than 0.05; this phrase will be considered to mean "nominally statistically significant" and will not imply a prespecified hypothesis test. Imputation of missing data is not planned.

This study is an extension of Study 101A. The results from Study 101A may be included in analyses of results from this study.

Analysis Sets

- Full Analysis Set (FAS): all patients who receive any study medication. This analysis set is consistent with the intent-to-treat (ITT) principle and will be used for safety analyses. This analysis set will include all Study 101A data for the patients who have received at least 1 dose of pegzilarginase in the current study.
- PK set: all patients in the FAS with quantifiable serum concentrations of pegzilarginase and who provide sufficient serum concentrations to support PK parameter estimation (in either Study 101A or the current study).

Patient Disposition

The number and percentage of patients entering and completing the clinical study will be presented, along with primary reason for discontinuation of any patients who discontinue prior to study termination.

Demographics and Baseline Characteristics

Demographics and baseline characteristics will be summarized from the data from Study 101A. A comparison of baseline characteristics from Study 101A that were reassessed during screening for the current study may be presented.

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Primary Endpoints (safety/tolerability)

All AEs will be listed and summarized. The number and percentage of patients with AEs and treatment-related AEs; SAEs and treatment-related SAEs; discontinuations due to an AE; and fatal AEs will be summarized by Medical Dictionary for Regulatory Activities (MedDRA) system organ class and preferred term. Summaries of AEs by maximum severity will also be produced.

Vital signs will be listed and summarized across time. Abnormal changes in ECGs and physical examinations will be listed.

Laboratory data will be listed and summarized across time; values outside the normal range will be flagged. Primary laboratory studies will include hematology, chemistry, urinalysis, coagulation, plasma arginine levels, and ammonia levels. Immunogenicity safety measures will include levels of ADA and anti-PEG antibodies. For any patients who develop low plasma arginine levels associated with clinically significant symptoms, clinically significant hyperammonemia, and or ADAs to either pegzilarginase or PEG, listings will be provided for temporally related AEs and laboratory data (arginine, ammonia, antibody titers, and PK data).

Secondary Endpoints

Plasma Arginine Levels

On-treatment arginine levels will be summarized. Each patient will serve as his/her own control. Arginine levels will also be listed across time. The relationship between arginine and other endpoints may be described graphically, such as with scatterplots. Additional mathematical modeling of arginine data may be performed.

Plasma Guanidino Compounds

Levels of guanidino compounds will be listed and summarized across time. Values outside the normal range will be flagged.

Pharmacokinetics

PK parameters to be evaluated are time of maximum observed concentration (t_{max}), maximum observed concentration (C_{max}), area under the curve through the time of the last measurable concentration (AUC^{0-t}), area under the curve extrapolated to infinity ($AUC_{0-\infty}$), AUC over the dosing interval (AUC_{0-168}), apparent terminal elimination half-life ($t_{½}$), serum clearance of drug per unit time (CL after IV dosing; CL/F after SC dosing), and volume of distribution (V_{ss} after IV dosing; Vz/F after SC dosing.

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Neurologic and Neuromotor Manifestations

Results from the neurologic and neuromotor testing results will be summarized; details will be provided in the SAP or in a separate related document.

Other Manifestations

Seizure frequency, frequency of hyperammonemic episodes, and incidence of disease-related transaminase elevations will be summarized; details will be provided in the SAP or in a separate related document.

Exploratory Endpoints

Details of the analysis of the exploratory endpoints (as listed above) will be described in the SAP or in a separate related document.

Power and Sample Size

The sample size of approximately 10 pediatric and adult patients was determined using clinical, rather than statistical, considerations. This sample size may lack power to distinguish a statistically significant result.

Table 1: Schedule of Assessments for Weeks 1 to 12 (IV)

			Weekly (QW) IV Dosing											
	Scr	**BL**[a]	**Wk 1**	**Wk 2**	**Wk 3**	**Wk 4**	**Wk 5**	**Wk 6**	**Wk 7**	**Wk 8**	**Wk 9**	**Wk 10**	**Wk 11**	**Wk 12**
Informed consent	X													
Inclusion/exclusion	X													
Medical history		X												
Height, weight[b]		X				X				X				X
Physical examination		X												X
ECG		X												X
Clinical laboratory tests[c]		X	X							X				
Complement C3 and tryptase[c]		X												
Pregnancy test[d]		X	X			X				X				X
3-day diet record[e]			X			X				X				X
Pegzilarginase dosing[f]			X	X	X	X	X	X	X	X	X	X	X	X
Arginine/ornithine[g]			X	X	X	X	X	X	X	X	X	X	X	X
Guanidino compounds[g]			X	X	X	X	X	X	X	X	X	X	X	X
PK profile[h]			X							X				
ALT/AST profiles, ammonia[i]		X	X			X				X				X
ADA[j]			X			X				X				X
Other amino acids (glu, val, leu, ile)			X											X
Neurological/neuromotor, adaptive behavior, and QoL assessments[k]		X												X
Neurocognitive and developmental assessments[l]		X												
Clinical growth assessments[m]		X												
Clinic/CRU visit[n]	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital signs[o]		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
Adverse events		X	X	X	X	X	X	X	X	X	X	X	X	X
Seizure frequency		X	X	X	X	X	X	X	X	X	X	X	X	X

ADA = anti-drug antibodies (anti-pegzilarginase antibodies); ALT = alanine aminotransferase; AST = aspartate aminotransferase; BL = baseline; CRU = clinical research unit; ECG = electrocardiogram; EOI = end of infusion; glu = glutamine; ile = isoleucine; IV= intravenous; leu = leucine; PEG = polyethylene glycol; PK = pharmacokinetic; Scr = screening; QoL = quality of life; QW = once weekly; val = valine; Wk = week.

NOTE: Efforts should be made to collect all blood samples where clinically practical. Samples collected after the end of infusion (IV dosing) should be collected within approximately 30 minutes. Deviations from blood sampling timepoints are allowable to accommodate patient or site scheduling. If a sample is unable to be collected, this should be noted in the patient's records but will not constitute a protocol deviation. The actual date and time of collection of all samples obtained must be recorded on the specimen sent to the laboratory and in the patient's source document and in the case report forms (CRFs).
NOTE: The protocol allows flexibility in dosing regimen after the first 12 weeks. The protocol allows that patients may stay on a regimen for more or less than 12 weeks and may switch between regimens as clinically indicated. The procedures and evaluations conducted at any visit will coincide with the above table based on the regimen the patient is assigned to at the time of the visit (with the exception of dosing at the site after the first twelve weeks and after the fourth SC dose).
Dark Gray Shading = predose, EOI, and postdose at 4 hours, 8 hours, 24 hours, 48 hours, 120 hours, and 168 hours (also occurs 8 weeks after any dose [mg/kg], regimen, or route-of-administration change).
Light Gray Shading = predose and 4 hours postdose.

[a] The Baseline visit should occur approximately 4 weeks (but no sooner than 3 weeks) after the last dose of study medication on the 101A study. The 101A study follow-up assessments may substitute for Study CAEB1102-102A Baseline assessments if the 101A follow up assessments were collected within 4 weeks of the Baseline visit of the 102A study. The Screening visit can occur at any time between the last dose on the 101A study and the Baseline visit on this study. The Screening and Baseline visits may occur on the same day as long as that day is at least 3 weeks after the last dose on the 101A study.
[b] Height will be collected at baseline for all patients and every 24 weeks thereafter for pediatric patients only. Weight will be obtained during the baseline period and approximately every 4 weeks during dosing. The baseline weight can be used to calculate each total dose, unless a subsequent weight obtained indicates a >10% change from the baseline weight.
[c] Clinical safety laboratory tests should be repeated at Week 1 if Week 1 is performed greater than 14 days from the baseline visit. Serum tryptase and serum complement C3 will also be obtained in the event of a hypersensitivity reaction. Samples should be obtained no later than 3 hours after the start of the reaction and at 24 hours after the start of the reaction.
[d] Pregnancy test can either be a serum or a urine test.
[e] Patients will complete a 3-day diet record using a diary to collect the information prior to dosing visit every 4 weeks beginning prior to the first dose at Week 1.
[f] See Section 9.7.1 for details regarding dosing, including frequency and route.
[g] Samples for arginine, ornithine, and guanidino compounds will be collected prior to dosing for every dose during the first 24 weeks or as clinically indicated. A predose sample must also be obtained before any dose or route-of-administration change and prior to the 3 subsequent doses at the new dose level. In addition, at Week 1, Week 8, Week 24, and 8 weeks after any dose change, sampling times will be predose, within 30 minutes after infusion (IV only), and postdose at approximately 4, 8, 24, 48, 120, and 168 hours. An approximate 2-hour fast should be observed prior to all sampling for amino acids and within 1 hour prior to dose, except where operationally and/or clinically not possible.
[h] PK samples for pegzilarginase will be collected at Week 1, Week 8, Week 24, and 8 weeks after any dose change. Sampling times will be predose, within 30 minutes after infusion (IV only), and postdose at approximately 4, 8, 24, 48, 120, and 168 hours. A predose sample will also be obtained before any dose or route-of-administration change and prior to the 3 subsequent doses at the new dose level.
[i] Samples for ALT/AST and ammonia will be collected at baseline, pre-dose and 4 hours post-dose every 4th dose for the first 24 weeks. In addition, at Week 1, Week 8, Week 24, and 8 weeks after any dose change, sampling times will be predose, within 30 minutes after infusion (IV only), and postdose at approximately 4, 8, 24, 48, 120, and 168 hours.
[j] Anti-drug antibodies (ADA) will be collected prior to dosing (within 1 hour of dose) at Week 1 then at every 4 weeks for the first 12 weeks, then every 8 weeks. ADA testing will include assessment of antibodies against pegzilarginase and against polyethylene glycol (PEG). An unscheduled sample for ADA assessment will be obtained in the event of a moderate or severe hypersensitivity reaction. Patients who test positive for ADA will be followed and re-tested approximately every 2 to 3 months after the end of the study until ADA levels return to baseline.
[k] Neurological/neuromotor, adaptive behavior, and QoL assessments will be collected at baseline, 12 weeks, and 24 weeks: 6-Minute Walk Test, Modified Ashworth Scale, Gross Motor Function Measure, Berg Balance Scale, Purdue Pegboard Test; Vineland Adaptive Behavior Scales II; PROMIS (for patients that began the 101A study utilizing a PROMIS test) or PedsQL Measurement Model for the Pediatric Quality of Life Inventory (for patients that did not begin the 101A study utilizing a PROMIS tool); Short Form

Zarit Burden Interview (ZBI-12). On days when a timed walk test is scheduled, it should be performed before any other neurological/neuromotor, developmental, or neurocognitive tests scheduled for that day. These assessments may be delayed up to 2 weeks to accommodate scheduling.

[l] Neurocognitive and developmental, assessments will be collected at baseline and 24 weeks using age appropriate assessments: Wechsler Adult Intelligence Scale IV; Wechsler Intelligence Scale for Children V; Wechsler Preschool and Primary Scale of Intelligence IV; Bayley Scales of Infant Development III; Rey Auditory Verbal Learning Test; Atlantis subtest on the Kaufman Assessment Battery for Children II; Test of Variables of Attention version 9. These assessments may be delayed up to 2 weeks to accommodate scheduling.

[m] Growth assessments will be performed for pediatric patients (ages 2 to 18): age-appropriate length/height, weight, head circumference measures.

[n] Admission to CRU and observation time in CRU post infusion will be the clinical decision of the Investigator. After Week 12, if appropriate in the opinion of the Investigator, home health care visits may be utilized. Patients will be required to return to the CRU at Week 24 or as clinically indicated in the opinion of the Investigator.

[o] Vital signs will be obtained within 60 min prior to dosing and within 10 min after the end of dosing.

Table 2: Schedule of Assessments for Weeks 13 to 24 (IV)

	Weekly (QW) IV Dosing											
	Wk 13	**Wk 14**	**Wk 15**	**Wk 16**	**Wk 17**	**Wk 18**	**Wk 19**	**Wk 20**	**Wk 21**	**Wk 22**	**Wk 23**	**Wk 24**
Height, weight[a]				X				X				X
Physical examination												X
ECG												X
Clinical laboratory tests				X								X
Complement C3 and tryptase[b]												
Pregnancy test[c]				X				X				X
3-day diet record[d]				X				X				X
Pegzilarginase dosing[e]	X	X	X	X	X	X	X	X	X	X	X	X
Arginine/ornithine[f]	X	X	X	X	X	X	X	X	X	X	X	X
Guanidino compounds[f]	X	X	X	X	X	X	X	X	X	X	X	X
PK profile[g]												X
ALT/AST profiles, ammonia[h]				X				X				X
ADA[i]								X				
Other amino acids (glu, val, leu, ile)												X
Neurological/neuromotor, adaptive behavior, and QoL assessments[j]												X
Neurocognitive and developmental assessments[k]												X
Clinical growth assessments[l]												X

	Weekly (QW) IV Dosing											
	Wk 13	**Wk 14**	**Wk 15**	**Wk 16**	**Wk 17**	**Wk 18**	**Wk 19**	**Wk 20**	**Wk 21**	**Wk 22**	**Wk 23**	**Wk 24**
Clinic/CRU visit[m]												X
Vital signs[n]	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
Adverse events	X	X	X	X	X	X	X	X	X	X	X	X
Seizure frequency	X	X	X	X	X	X	X	X	X	X	X	X

ADA = anti-drug antibodies (anti-pegzilarginase antibodies); ALT = alanine aminotransferase; AST = aspartate aminotransferase; BL = baseline; CRU = clinical research unit; ECG = electrocardiogram; EOI = end of infusion; glu = glutamine; ile = isoleucine; IV= intravenous; leu = leucine; PEG = polyethylene glycol; PK = pharmacokinetic; Scr = screening; QoL = quality of life; QW = once weekly; val = valine; Wk = week.

NOTE: The protocol allows flexibility in dosing regimen after the first 12 weeks. The protocol allows that patients may stay on a regimen for more or less than 12 weeks and may switch between regimens as clinically indicated. The procedures and evaluations conducted at any visit will coincide with the above table based on the regimen the patient is assigned to at the time of the visit (with the exception of dosing at the site after the first twelve weeks and after the fourth SC dose).

Dark Gray Shading = predose, EOI, and postdose at 4 hours, 8 hours, 24 hours, 48 hours, 120 hours, and 168 hours (also occurs 8 weeks after any dose [mg/kg], regimen, or route-of-administration change route change).

Light Gray Shading = predose and 4 hours postdose.

[a] Height will be collected at baseline for all patients and every 24 weeks thereafter for pediatric patients only. Weight will be obtained during the baseline period and approximately every 4 weeks during dosing. The baseline weight can be used to calculate each total dose, unless a subsequent weight obtained indicates a >10% change from the baseline weight.

[b] Clinical safety laboratory tests should be repeated at Week 1 if Week 1 is performed greater than 14 days from the baseline visit. Serum tryptase and serum complement C3 will also be obtained in the event of a hypersensitivity reaction. Samples should be obtained no later than 3 hours after the start of the reaction and at 24 hours after the start of the reaction.

[c] Pregnancy test can either be a serum or a urine test.

[d] Patients will complete a 3-day diet record using a diary to collect the information prior to dosing visit every 4 weeks beginning prior to the first dose at Week 1.

[e] See Section 9.7.1 for details regarding dosing, including frequency and route.

[f] Samples for arginine, ornithine, and guanidino compounds will be collected prior to dosing for every dose during the first 24 weeks or as clinically indicated. A predose sample must also be obtained before any dose or route-of-administration change and prior to the 3 subsequent doses at the new dose level. In addition, at Week 1, Week 8, Week 24, and 8 weeks after any dose change, sampling times will be predose, within 30 minutes after infusion (IV only), and postdose at approximately 4, 8, 24, 48, 120, and 168 hours. An approximate 2-hour fast should be observed prior to all sampling for amino acids and within 1 hour prior to dose, except where operationally and /or clinically not possible.

[g] PK samples for pegzilarginase will be collected at Week 1, Week 8, Week 24, and 8 weeks after any dose change. Sampling times will be predose, within 30 minutes after infusion (IV only), and postdose at approximately 4, 8, 24, 48, 120, and 168 hours. A predose sample will also be obtained before any dose or route-of-administration change and prior to the 3 subsequent doses at the new dose level.

[h] Samples for ALT/AST and ammonia will be collected at baseline, pre-dose and 4 hours post-dose every 4th dose for the first 24 weeks. In addition, at Week 1, Week 8, Week 24, and 8 weeks after any dose change, sampling times will be predose, within 30 minutes after infusion (IV only), and postdose at approximately 4, 8, 24, 48, 120, and 168 hours.

[i] Anti-drug antibodies (ADA) will be collected prior to dosing (within 1 hour of dose) at Week 1 then at every 4 weeks for the first 12 weeks, then every 8 weeks. ADA testing will include assessment of antibodies against pegzilarginase and against polyethylene glycol (PEG). An unscheduled sample for ADA assessment will be obtained in the event of a moderate or severe hypersensitivity reaction. Patients who test positive for ADA will be followed and re-tested approximately every 2 to 3 months after the end of the study until ADA levels return to baseline.

[j] Neurological/neuromotor, adaptive behavior, and QoL assessments will be collected at baseline, 12 weeks, and 24 weeks: 6-Minute Walk Test, Modified Ashworth Scale, Gross Motor Function Measure, Berg Balance Scale, Purdue Pegboard Test; Vineland Adaptive Behavior Scales II; PROMIS (for patients that began the 101A study utilizing a PROMIS test) or PedsQL Measurement Model for the Pediatric Quality of Life Inventory (for patients that did not begin the 101A study utilizing a PROMIS tool); Short Form Zarit Burden Interview (ZBI-12). On days when a timed walk test is scheduled, it should be performed before any other neurological/neuromotor, developmental, or neurocognitive tests scheduled for that day. These assessments may be delayed up to 2 weeks to accommodate scheduling.

[k] Neurocognitive and developmental, assessments will be collected at baseline and 24 weeks using age appropriate assessments: Wechsler Adult Intelligence Scale IV; Wechsler Intelligence Scale for Children V; Wechsler Preschool and Primary Scale of Intelligence IV; Bayley Scales of Infant Development III; Rey Auditory Verbal Learning Test; Atlantis subtest on the Kaufman Assessment Battery for Children II; Test of Variables of Attention version 9. These assessments may be delayed up to 2 weeks to accommodate scheduling.

[l] Growth assessments will be performed for pediatric patients (ages 2 to 18): age-appropriate length/height, weight, head circumference measures.

[m] Admission to CRU and observation time in CRU post infusion will be the clinical decision of the Investigator. After Week 12, if appropriate in the opinion of the Investigator, home health care visits may be utilized. Patients will be required to return to the CRU at Week 24 or as clinically indicated in the opinion of the Investigator.

[n] Vital signs will be obtained within 60 min prior to dosing and within 10 min after the end of dosing.

Table 3: Schedule of Assessments for Weeks 25 to 37 (SC)

	Weekly (QW) SC Dosing												
	Wk 25	**Wk 26**	**Wk 27**	**Wk 28**	**Wk 29**	**Wk 30**	**Wk 31**	**Wk 32**	**Wk 33**	**Wk 34**	**Wk 35**	**Wk 36**	**Wk 37**
Height, weight[a]				X				X				X	
ECG												X	
Clinical laboratory tests								X					
Complement C3 and tryptase[b]													
Pregnancy test[c]				X				X				X	
3-day diet record[d]				X				X				X	
Pegzilarginase dosing[e]	X	X	X	X	X	X	X	X	X	X	X	X	X
Arginine/ornithine[f]	X	X	X	X		X		X				X	
Guanidino compounds[f]	X	X	X	X		X		X				X	
PK profile[g]	X	X	X	X				X				X	
Additional PK[h]													
ALT/AST profiles, ammonia[i]								X				X	
ADA[j]				X								X	
Other amino acids (glu, val, leu, ile)												X	
Neurological/neuromotor, adaptive behavior, and QoL assessments[k]												X	

	Weekly (QW) SC Dosing												
	Wk 25	**Wk 26**	**Wk 27**	**Wk 28**	**Wk 29**	**Wk 30**	**Wk 31**	**Wk 32**	**Wk 33**	**Wk 34**	**Wk 35**	**Wk 36**	**Wk 37**
Neurocognitive and developmental assessments[l]													
Clinical growth assessments[m]													
Clinic/CRU visit[n]	X	X	X	X								X	
Vital signs[o]	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
Adverse events	X	X	X	X	X	X	X	X	X	X	X	X	X
Seizure frequency		X	X	X	X	X	X	X	X	X	X	X	X

ADA = anti-drug antibodies (anti-pegzilarginase antibodies); ALT = alanine aminotransferase; AST = aspartate aminotransferase; CRU = clinical research unit; ECG = electrocardiogram; glu = glutamine; ile = isoleucine; IV = intravenous; leu = leucine; PEG = polyethylene glycol; PK = pharmacokinetic; SC = subcutaneous; QoL = quality of life; QW = once weekly; val = valine; Wk = week.

NOTE: Efforts should be made to collect all blood samples where clinically practical. Samples collected after the end of infusion (IV dosing) should be collected within approximately 30 minutes. Deviations from blood sampling timepoints are allowable to accommodate patient or site scheduling. If a sample is unable to be collected, this should be noted in the patient's records but will not constitute a protocol deviation. The actual date and time of collection of all samples obtained must be recorded on the specimen sent to the laboratory and in the patient's source document and in the case report forms (CRFs).

NOTE: The protocol allows flexibility in dosing regimen after the first 12 weeks. The protocol allows that patients may stay on a regimen for more or less than 12 weeks and may switch between regimens as clinically indicated. The procedures and evaluations conducted at any visit will coincide with the above table based on the regimen the patient is assigned to at the time of the visit (with the exception of dosing at the site after the first twelve weeks and after the fourth SC dose).

Dark Gray Shading = predose, and postdose at 4 hours, 8 hours, 24 hours, 48 hours, 120 hours, and 168 hours (also occurs 8 weeks after any dose [mg/kg], regimen, or route-of-administration change).

Light Gray Shading = predose and 4 hours postdose.

[a] Height will be collected every 24 weeks for pediatric patients only. Weight will be obtained during the baseline period and approximately every 4 weeks during dosing. The baseline weight can be used to calculate each total dose, unless a subsequent weight obtained indicates a > 10% change from the baseline weight.

[b] Serum tryptase and serum complement C3 will be obtained in the event of a hypersensitivity reaction. Samples should be obtained no later than 3 hours after the start of the reaction and at 24 hours after the start of the reaction.

[c] Pregnancy test can either be a serum or a urine test.

[d] Patients will complete a 3-day diet record using a diary to collect the information prior to dosing visit every 4 weeks beginning prior to the first dose at Week 1.

[e] See Section 9.7.1 for details regarding dosing, including frequency and route.

[f] Samples for arginine, ornithine, and guanidino compounds will be collected prior to dosing every second dose or as clinically indicated. A predose sample must also be obtained before any dose or route-of-administration change and prior to the 3 subsequent doses at the new level. In addition, at 8 weeks after any dose or route-of-administration change, sampling times will be predose, within 30 minutes after infusion (IV only), and postdose at approximately 4, 8, 24, 48, 120, and 168 hours. An approximate 2-hour fast should be observed prior to all sampling for amino acids and within 1 hour prior to dose, except where operationally and/or clinically not possible.

[g] PK samples for pegzilarginase will be collected at the start of SC dosing (Wk 25) and 8 weeks after any dose or route-of-administration change. Sampling times will be predose, within 30 minutes after infusion (IV only), and postdose at approximately 4, 8, 24, 48, 120, and 168 hours. A predose sample will also be obtained before any dose or route-of-administration change and prior to the 3 subsequent doses at the new dose level.

[h] Additional PK samples for pegzilarginase will be collected during SC dosing after the patient has received a minimum of 4 consecutive doses of pegzilarginase with no missed doses or changes in dose levels. This PK sampling will occur at the subject's next regularly scheduled visit in which neuromotor and/or neurocognitive assessments are conducted, based on the subject's availability for repeat samples. If it is not feasible to obtain this additional PK profile at the next neurocognitive or neuromotor assessment visit, additional PK samples may be taken during the course of any other visit. Sampling times will be predose, postdose at approximately 4, 8, 24, 48, 72, 96, 120, 144, and 168 hours. This PK sampling may be repeated 8 weeks later if a subject misses any sampling timepoint. An additional ADA sample will be taken at the 168-hour timepoint of this additional PK sampling.

[i] Samples for ALT/AST and ammonia will be collected pre-dose and 4 hours post-dose every 12 weeks. In addition, at 8 weeks after any dose or route-of-administration change, sampling times will be predose, within 30 minutes after infusion (IV only), and postdose at approximately 4, 8, 24, 48, 120, and 168 hours.

[j] Anti-drug antibodies (ADA) will be collected prior to dosing (within 1 hour of dose) every 8 weeks until Week 36, then approximately every 12 weeks during the dosing period, and at follow-up. ADA testing will include assessment of antibodies against pegzilarginase and against polyethylene glycol (PEG). An unscheduled sample for ADA assessment will be obtained in the event of a moderate or severe hypersensitivity reaction. Patients who test positive for ADA will be followed and re-tested approximately every 2 to 3 months after the end of the study until ADA levels return to baseline. An additional ADA sample will be taken at the 168-hour timepoint of the additional PK sampling.

[k] Neurological/neuromotor, adaptive behavior, and QoL assessments will be collected at 36 and 48 weeks: 6-Minute Walk Test, Modified Ashworth Scale, Gross Motor Function Measure, Berg Balance Scale, Purdue Pegboard Test; Vineland Adaptive Behavior Scales II; PROMIS (for patients that began the 101A study utilizing a PROMIS test) or PedsQL Measurement Model for the Pediatric Quality of Life Inventory (for patients that did not begin the 101A study utilizing a PROMIS tool); Short Form Zarit Burden Interview (ZBI-12). On days when a timed walk test is scheduled, it should be performed before any other neurological/neuromotor, developmental, or neurocognitive tests scheduled for that day. These assessments may be delayed up to 2 weeks to accommodate scheduling.

[l] Neurocognitive and developmental, assessments will be collected at 48 weeks using age appropriate assessments: Wechsler Adult Intelligence Scale IV; Wechsler Intelligence Scale for Children V; Wechsler Preschool and Primary Scale of Intelligence IV; Bayley Scales of Infant Development III; Rey Auditory Verbal Learning Test; Atlantis subtest on the Kaufman Assessment Battery for Children II; Test of Variables of Attention version 9. These assessments may be delayed up to 2 weeks to accommodate scheduling.

[m] Growth assessments will be performed for pediatric patients (ages 2 to 18): age-appropriate length/height, weight, head circumference measures.

[n] Admission to CRU and observation time in CRU post infusion will be the clinical decision of the Investigator. It is recommended that if patients are switched to SC dosing, at least the first 4 SC doses be administered in the clinic (Weeks 25, 26, 27, and 28). After Week 28, if appropriate in the opinion of the Investigator, home health care visits may be utilized. Patients will be required to return to the CRU at Week 36 and Week 48 or as clinically indicated in the opinion of the Investigator.

[o] Vital signs will be obtained within 60 min prior to dosing and within 10 min after the end of dosing.

Table 4: Schedule of Assessments for Weeks 38 to 48 (SC)

	Weekly (QW) SC Dosing										
	Wk 38	**Wk 39**	**Wk 40**	**Wk 41**	**Wk 42**	**Wk 43**	**Wk 44**	**Wk 45**	**Wk 46**	**Wk 47**	**Wk 48**
Height, weight[a]			X				X				X
Physical examination											X
ECG											X
Clinical laboratory tests			X								X
Complement C3 and tryptase[b]											
Pregnancy test[c]			X				X				X
3-day diet record[d]			X				X				X
Pegzilarginase dosing[e]	X	X	X	X	X	X	X	X	X	X	X

	Weekly (QW) SC Dosing										
	Wk 38	**Wk 39**	**Wk 40**	**Wk 41**	**Wk 42**	**Wk 43**	**Wk 44**	**Wk 45**	**Wk 46**	**Wk 47**	**Wk 48**
Arginine/ornithine[f]	X		X		X		X		X		X
Guanidino compounds[f]	X		X		X		X		X		X
PK profile[g]											
Additional PK[h]											
ALT/AST profiles, ammonia[i]											X
ADA[j]											X
Other amino acids (glu, val, leu, ile)											X
Neurological/neuromotor, adaptive behavior, and QoL assessments[k]											X
Neurocognitive and developmental assessments[l]											X
Clinical growth assessments[m]											X
Clinic/CRU visit[n]											X
Vital signs[o]	X	X	X	X	X	X	X	X	X	X	X
Concomitant medications	X	X	X	X	X	X	X	X	X	X	X
Adverse events	X	X	X	X	X	X	X	X	X	X	X
Seizure frequency	X	X	X	X	X	X	X	X	X	X	X

ADA = anti-drug antibodies (anti-pegzilarginase antibodies); ALT = alanine aminotransferase; AST = aspartate aminotransferase; CRU = clinical research unit; ECG = electrocardiogram; glu = glutamine; ile = isoleucine; IV = intravenous; leu = leucine; PEG = polyethylene glycol; PK = pharmacokinetic; SC = subcutaneous; QoL = quality of life; QW = once weekly; val = valine; Wk = week.

NOTE: Efforts should be made to collect all blood samples where clinically practical. Samples collected after the end of infusion (IV dosing) should be collected within approximately 30 minutes. Deviations from blood sampling timepoints are allowable to accommodate patient or site scheduling. If a sample is unable to be collected, this should be noted in the patient's records but will not constitute a protocol deviation. The actual date and time of collection of all samples obtained must be recorded on the specimen sent to the laboratory and in the patient's source document and in the case report forms (CRFs).

NOTE: The protocol allows flexibility in dosing regimen after the first 12 weeks. The protocol allows that patients may stay on a regimen for more or less than 12 weeks and may switch between regimens as clinically indicated. The procedures and evaluations conducted at any visit will coincide with the above table based on the regimen the patient is assigned to at the time of the visit (with the exception of dosing at the site after the first twelve weeks and after the fourth SC dose).

Light Gray Shading = predose and 4 hours postdose

[a] Height will be collected every 24 weeks for pediatric patients only. Weight will be obtained during the baseline period and approximately every 4 weeks during dosing. The baseline weight can be used to calculate each total dose, unless a subsequent weight obtained indicates a > 10% change from the baseline weight.

[b] Serum tryptase and serum complement C3 will be obtained in the event of a hypersensitivity reaction. Samples should be obtained no later than 3 hours after the start of the reaction and at 24 hours after the start of the reaction.

[c] Pregnancy test can either be a serum or a urine test.
[d] Patients will complete a 3-day diet record using a diary to collect the information prior to dosing visit every 4 weeks beginning prior to the first dose at Week 1.
[e] See Section 9.7.1 for details regarding dosing, including frequency and route.
[f] Samples for arginine, ornithine, and guanidino compounds will be collected prior to dosing every second dose or as clinically indicated. A predose sample must also be obtained before any dose or route-of-administration change and prior to the 3 subsequent doses at the new level. In addition, at 8 weeks after any dose or route-of-administration change, sampling times will be predose, within 30 minutes after infusion (IV only), and postdose at approximately 4, 8, 24, 48, 120, and 168 hours. An approximate 2-hour fast should be observed prior to all sampling for amino acids and within 1 hour prior to dose, except where operationally and/or clinically not possible.
[g] PK samples for pegzilarginase will be collected at the start of SC dosing (Wk 25) and 8 weeks after any dose change. Sampling times will be predose, within 30 minutes after infusion (IV only), and postdose at approximately 4, 8, 24, 48, 120, and 168 hours. A predose sample will also be obtained before any dose or route-of-administration change and prior to the 3 subsequent doses at the new dose level.
[h] Additional PK samples for pegzilarginase will be collected during SC dosing after the patient has received a minimum of 4 consecutive doses of pegzilarginase with no missed doses or changes in dose levels. This PK sampling will occur at the subject's next regularly scheduled visit in which neuromotor and/or neurocognitive assessments are conducted, based on the subject's availability for repeat samples. Sampling times will be predose, postdose at approximately 4, 8, 24, 48, 72, 96, 120, 144, and 168 hours. This PK sampling may be repeated 8 weeks later if a subject misses any sampling timepoint. An additional ADA sample will be taken at the 168 hour timepoint
[i] Samples for ALT/AST and ammonia will be collected pre-dose and 4 hours post-dose every 12 weeks. In addition, at 8 weeks after any dose or route-of-administration change, sampling times will be predose, within 30 minutes after infusion (IV only), and postdose at approximately 4, 8, 24, 48, 120, and 168 hours.
[j] Anti-drug antibodies (ADA) will be collected prior to dosing (within 1 hour of dose) every 8 weeks until Week 36, then approximately every 12 weeks during the dosing period, and at follow-up. ADA testing will include assessment of antibodies against pegzilarginase and against polyethylene glycol (PEG). An unscheduled sample for ADA assessment will be obtained in the event of a moderate or severe hypersensitivity reaction. Patients who test positive for ADA will be followed and re-tested approximately every 2 to 3 months after the end of the study until ADA levels return to baseline. An additional ADA sample will be taken at the 168-hour timepoint of the additional PK sampling.
[k] Neurological/neuromotor, adaptive behavior, and QoL assessments will be collected at 36 and 48 weeks: 6-Minute Walk Test, Modified Ashworth Scale, Gross Motor Function Measure, Berg Balance Scale, Purdue Pegboard Test; Vineland Adaptive Behavior Scales II; PROMIS (for patients that began the 101A study utilizing a PROMIS test) or PedsQL Measurement Model for the Pediatric Quality of Life Inventory (for patients that did not begin the 101A study utilizing a PROMIS tool); Short Form Zarit Burden Interview (ZBI-12). On days when a timed walk test is scheduled, it should be performed before any other neurological/neuromotor, developmental, or neurocognitive tests scheduled for that day. These assessments may be delayed up to 2 weeks to accommodate scheduling.
[l] Neurocognitive and developmental, assessments will be collected at 48 weeks using age appropriate assessments: Wechsler Adult Intelligence Scale IV; Wechsler Intelligence Scale for Children V; Wechsler Preschool and Primary Scale of Intelligence IV; Bayley Scales of Infant Development III; Rey Auditory Verbal Learning Test; Atlantis subtest on the Kaufman Assessment Battery for Children II; Test of Variables of Attention version 9. These assessments may be delayed up to 2 weeks to accommodate scheduling.
[m] Growth assessments will be performed for pediatric patients (ages 2 to 18): age-appropriate length/height, weight, head circumference measures.
[n] Admission to CRU and observation time in CRU post infusion will be the clinical decision of the Investigator. It is recommended that if patients are switched to SC dosing, at least the first 4 SC doses be administered in the clinic (Weeks 25, 26, 27, and 28). After Week 28, if appropriate in the opinion of the Investigator, home health care visits may be utilized. Patients will be required to return to the CRU at Week 36 and Week 48 or as clinically indicated in the opinion of the Investigator.
[o] Vital signs will be obtained within 60 min prior to dosing and within 10 min after the end of dosing (for either IV or SC dosing).

Table 5: Schedule of Assessments for Weeks 49 to 104

	Weekly (QW) Dosing													
	Wk 52	**Wk 56**	**Wk 60**	**Wk 64**	**Wk 68**	**Wk 72**	**Wk 76**	**Wk 80**	**Wk 84**	**Wk 88**	**Wk 92**	**Wk 96**	**Wk 100**	**Wk 104**
Height, weight[a]	X	X	X	X	X	X	X	X	X	X	X	X		
Physical examination						X						X		

	Weekly (QW) Dosing													
	Wk 52	**Wk 56**	**Wk 60**	**Wk 64**	**Wk 68**	**Wk 72**	**Wk 76**	**Wk 80**	**Wk 84**	**Wk 88**	**Wk 92**	**Wk 96**	**Wk 100**	**Wk 104**
ECG												X		
Clinical laboratory tests		X		X		X		X		X		X		
Complement C3 and tryptase[b]														
Pregnancy test[c]	X	X	X	X	X	X	X	X	X	X	X	X	X	X
3-day diet record[d]	X	X	X	X	X	X	X	X	X	X	X	X		
Pegzilarginase dosing[e]	Weekly													
Arginine/ornithine[f]	Every other week													
Guanidino compounds[f]	Every other week													
PK profile[g]														
Additional PK[h]														
ALT/AST profiles, ammonia[i]			X			X			X			X		
ADA[j]			X			X			X			X		
Other amino acids (glu, val, leu, ile)			X			X			X			X		
Neurological/neuromotor, adaptive behavior, and QoL assessments[k]						X						X		
Neurocognitive and developmental assessments[l]						X						X		
Clinical growth assessments[m]						X						X		
Clinic/CRU visit[n]			X			X			X			X		
Vital signs[o]	Weekly													
Concomitant medications	Weekly													
Adverse events	Weekly													
Seizure frequency	Weekly													

ADA = anti-drug antibodies (anti-pegzilarginase antibodies); ALT = alanine aminotransferase; AST = aspartate aminotransferase; CRU = clinical research unit; ECG = electrocardiogram; glu = glutamine; ile = isoleucine; IV = intravenous; leu = leucine; PEG = polyethylene glycol; PK = pharmacokinetic; SC = subcutaneous; QoL = quality of life; QW = once weekly; val = valine; Wk = week.

NOTE: Efforts should be made to collect all blood samples where clinically practical. Samples collected after the end of infusion (IV dosing) should be collected within approximately 30 minutes. Deviations from blood sampling timepoints are allowable to accommodate patient or site scheduling. If a sample is unable to be collected, this should be noted in the patient's records but will not constitute a protocol deviation. The actual date and time of collection of all samples obtained must be recorded on the specimen sent to the laboratory and in the patient's source document and in the case report forms (CRFs).
NOTE: The protocol allows flexibility in dosing regimen after the first 12 weeks. The protocol allows that patients may stay on a regimen for more or less than 12 weeks and may switch between regimens as clinically indicated. The procedures and evaluations conducted at any visit will coincide with the above table

based on the regimen the patient is assigned to at the time of the visit (with the exception of dosing at the site after the first twelve weeks and after the fourth SC dose).
Light Gray Shading = predose and 4 hours postdose

[a] Height will be collected every 24 weeks for pediatric patients only. Weight will be obtained during the baseline period and approximately every 4 weeks during dosing. The baseline weight can be used to calculate each total dose, unless a subsequent weight obtained indicates a > 10% change from the baseline weight.
[b] Serum tryptase and serum complement C3 will be obtained in the event of a hypersensitivity reaction. Samples should be obtained no later than 3 hours after the start of the reaction and at 24 hours after the start of the reaction.
[c] Pregnancy test can either be a serum or a urine test.
[d] Patients will complete a 3-day diet record using a diary to collect the information prior to dosing visit every 4 weeks beginning prior to the first dose at Week 1.
[e] See Section 9.7.1 for details regarding dosing, including frequency and route.
[f] Samples for arginine, ornithine, and guanidino compounds will be collected prior to dosing every second dose or as clinically indicated. A predose sample must also be obtained before any dose or route change and prior to the 3 subsequent doses at the new level. In addition, at 8 weeks after any dose or route-of-administration change, sampling times will be predose, within 30 minutes after infusion (IV only), and postdose at approximately 4, 8, 24, 48, 120, and 168 hours. An approximate 2-hour fast should be observed prior to all sampling for amino acids and within 1 hour prior to dose, except where operationally and/or clinically not possible.
[g] PK samples for pegzilarginase will be collected at the start of SC dosing (Wk 25) and 8 weeks after any dose change. Sampling times will be predose, within 30 minutes after infusion (IV only), and postdose at approximately 4, 8, 24, 48, 120, and 168 hours. A predose sample will also be obtained before any dose or route-of-administration change and prior to the 3 subsequent doses at the new dose level.
[h] Additional PK samples for pegzilarginase will be collected during SC dosing after the subject has received a minimum of 4 consecutive doses of pegzilarginase with no missed doses or changes in dose levels. This PK sampling will occur at the subject's next regularly scheduled visit in which neuromotor and/or neurocognitive assessments are conducted, based on the subject's availability for repeat samples. Sampling times will be predose, postdose at approximately 4, 8, 24, 48, 72, 96, 120, 144, and 168 hours. This PK sampling may be repeated 8 weeks later if a subject misses any sampling timepoint. An additional ADA sample will be taken at the 168-hour timepoint.
[i] Samples for ALT/AST and ammonia will be collected pre-dose and 4 hours post-dose every 12 weeks. In addition, at 8 weeks after any dose or route-of-administration change, sampling times will be predose, within 30 minutes after infusion (IV only), and postdose at approximately 4, 8, 24, 48, 120, and 168 hours.
[j] Anti-drug antibodies (ADA) will be collected prior to dosing (within 1 hour of dose) every 8 weeks until Week 36, then approximately every 12 weeks during the dosing period, and at follow-up. ADA testing will include assessment of antibodies against pegzilarginase and against polyethylene glycol (PEG). An unscheduled sample for ADA assessment will be obtained in the event of a moderate or severe hypersensitivity reaction. Patients who test positive for ADA will be followed and re-tested approximately every 2 to 3 months after the end of the study until ADA levels return to baseline. An additional ADA sample will be taken at the 168-hour timepoint of the additional PK sampling.
[k] Neurological/neuromotor, adaptive behavior, and QoL assessments will be collected at 72 and 96 weeks: 6-Minute Walk Test, Modified Ashworth Scale, Gross Motor Function Measure, Berg Balance Scale, Purdue Pegboard Test; Vineland Adaptive Behavior Scales II; PROMIS (for patients that began the 101A study utilizing a PROMIS test) or PedsQL Measurement Model for the Pediatric Quality of Life Inventory (for patients that did not begin the 101A study utilizing a PROMIS tool); Short Form Zarit Burden Interview (ZBI-12). On days when a timed walk test is scheduled, it should be performed before any other neurological/neuromotor, developmental, or neurocognitive tests scheduled for that day. These assessments may be delayed up to 2 weeks to accommodate scheduling.
[l] Neurocognitive and developmental, assessments will be collected at 72 and 96 weeks using age-appropriate assessments: Wechsler Adult Intelligence Scale IV; Wechsler Intelligence Scale for Children V; Wechsler Preschool and Primary Scale of Intelligence IV; Bayley Scales of Infant Development III; Rey Auditory Verbal Learning Test; Atlantis subtest on the Kaufman Assessment Battery for Children II; Test of Variables of Attention version 9. These assessments may be delayed up to 2 weeks to accommodate scheduling.
[m] Growth assessments will be performed for pediatric patients (ages 2 to 18): age-appropriate length/height, weight, head circumference measures.
[n] Admission to CRU and observation time in CRU post infusion will be the clinical decision of the Investigator. After Week 28, if appropriate in the opinion of the Investigator, home health care visits may be utilized. Patients will be required to return to the CRU every 12 weeks or as clinically indicated in the opinion of the Investigator.
[o] Vital signs will be obtained within 60 min prior to dosing and within 10 min after the end of dosing (for either IV or SC dosing) until Week 96. Beginning at Week 97, vital signs will be collected pre-dose only.

Table 6: Schedule of Assessments for Weeks 105 to 144

	Weekly (QW) Dosing									
	Wk 108	**Wk 112**	**Wk 116**	**Wk 120**	**Wk 124**	**Wk 128**	**Wk 132**	**Wk 136**	**Wk 140**	**Wk 144**
Height[a]				X						X
Weight[b]	X			X			X			X
Physical examination										X
ECG										X
Clinical laboratory tests	X			X			X			X
Complement C3 and tryptase[c]										
Pregnancy test[d]	X	X	X	X	X	X	X	X	X	X
3-day diet record[e]	X			X			X			X
Pegzilarginase dosing[f]	Weekly									
Arginine/ornithine[g]	X			X			X			X
Guanidino compounds[g]	X			X			X			X
Additional PK[h]										
ADA[i]	X			X			X			X
Other amino acids (glu, val, leu, ile)	X			X			X			X
Neurological/neuromotor, adaptive behavior, and QoL assessments[j]				X[k]						X
Neurocognitive and developmental assessments[l]										X
Clinical growth assessments[m]				X						X
Clinic/CRU visit[n]				X						X
Vital signs[o]	Weekly									
Concomitant medications	Weekly									
Adverse events	Weekly									
Seizure frequency	Weekly									

ADA = anti-drug antibodies (anti-pegzilarginase antibodies); ALT = alanine aminotransferase; AST = aspartate aminotransferase; CRU = clinical research unit; ECG = electrocardiogram; glu = glutamine; ile = isoleucine; IV = intravenous; leu = leucine; PEG = polyethylene glycol; PK = pharmacokinetic; SC = subcutaneous; QoL = quality of life; QW = once weekly; val = valine; Wk = week.

NOTE: Efforts should be made to collect all blood samples where clinically practical. Samples collected after the end of infusion (IV dosing) should be collected within approximately 30 minutes. Deviations from blood sampling timepoints are allowable to accommodate patient or site scheduling. If a sample is unable to be collected, this should be noted in the patient's records but will not constitute a protocol deviation. The actual date and time of collection of all samples obtained must be recorded on the specimen sent to the laboratory and in the patient's source document and in the case report forms (CRFs).

NOTE: The protocol allows flexibility in dosing regimen after the first 12 weeks. The protocol allows that patients may stay on a regimen for more or less than 12 weeks and may switch between regimens as clinically indicated. The procedures and evaluations conducted at any visit will coincide with the above table based on the regimen the patient is assigned to at the time of the visit (with the exception of dosing at the site after the first twelve weeks and after the fourth SC dose).

[a] Height will be collected every 24 weeks for pediatric patients only.
[b] Weight will be obtained during the baseline period and approximately every 12 weeks during dosing starting at Week 96. The baseline weight can be used to calculate each total dose, unless a subsequent weight obtained indicates a > 10% change from the baseline weight.
[c] Serum tryptase and serum complement C3 will be obtained in the event of a hypersensitivity reaction. Samples should be obtained no later than 3 hours after the start of the reaction and at 24 hours after the start of the reaction.
[d] Pregnancy test can either be a serum or a urine test.
[e] Patients will complete a 3-day diet record using a diary to collect the information prior to dosing visit every 12 weeks beginning at Week 96.
[f] See Section 9.7.1 for details regarding dosing, including frequency and route.
[g] Samples for arginine, ornithine, and guanidino compounds will be collected prior to dosing every 12 weeks or as clinically indicated starting at Week 96. A predose sample must also be obtained before any dose or route change and prior to the 3 subsequent doses at the new level. In addition, at 8 weeks after any dose or route-of-administration change, sampling times will be predose, within 30 minutes after infusion (IV only), and postdose at approximately 4, 8, 24, 48, 120, and 168 hours. An approximate 2-hour fast should be observed prior to all sampling for amino acids and within 1 hour prior to dose, except where operationally and/or clinically not possible. Samples will be collected via venipuncture or via fingerstick once a dry blood spot (DBS) becomes available.
[h] Additional PK samples for pegzilarginase will be collected during SC dosing after the patient has received a minimum of 4 consecutive doses of pegzilarginase with no missed doses or changes in dose levels. This PK sampling will occur at the subject's next regularly scheduled visit in which neuromotor and/or neurocognitive assessments are conducted, based on the subject's availability for repeat samples. Sampling times will be predose, postdose at approximately 4, 8, 24, 48, 72, 96, 120, 144, and 168 hours. This PK sampling may be repeated 8 weeks later if a subject misses any sampling timepoint. An additional ADA sample will be taken at the 168-hour timepoint.
[i] Anti-drug antibodies (ADA) will be collected prior to dosing (within 1 hour of dose) every 8 weeks until Week 36, then approximately every 12 weeks during the dosing period, and at follow-up. ADA testing will include assessment of antibodies against pegzilarginase and against polyethylene glycol (PEG). An unscheduled sample for ADA assessment will be obtained in the event of a moderate or severe hypersensitivity reaction. Patients who test positive for ADA will be followed and re-tested approximately every 2 to 3 months after the end of the study until ADA levels return to baseline. An additional ADA sample will be taken at the 168-hour timepoint of the additional PK sampling.
[j] Neurological/neuromotor, adaptive behavior, and QoL assessments will be collected: 6-Minute Walk Test, Modified Ashworth Scale, Gross Motor Function Measure, Berg Balance Scale, Purdue Pegboard Test; Vineland Adaptive Behavior Scales II; PROMIS (for patients that began the 101A study utilizing a PROMIS test) or PedsQL Measurement Model for the Pediatric Quality of Life Inventory (for patients that did not begin the 101A study utilizing a PROMIS tool); Short Form Zarit Burden Interview (ZBI-12). On days when a timed walk test is scheduled, it should be performed before any other neurological/neuromotor, developmental, or neurocognitive tests scheduled for that day. These assessments may be delayed up to 2 weeks to accommodate scheduling.
[k] At Week 120, only 3 neuromotor assessments (6MWT, GMFM-D, and GMFM-E) will be performed.
[l] Neurocognitive and developmental, assessments will be collected using age appropriate assessments: Wechsler Adult Intelligence Scale IV; Wechsler Intelligence Scale for Children V; Wechsler Preschool and Primary Scale of Intelligence IV; Bayley Scales of Infant Development III; Rey Auditory Verbal Learning Test; Atlantis subtest on the Kaufman Assessment Battery for Children II; Test of Variables of Attention version 9. These assessments may be delayed up to 2 weeks to accommodate scheduling.
[m] Growth assessments will be performed for pediatric patients (ages 2 to 18): age-appropriate length/height, weight, head circumference measures.
[n] Admission to CRU and observation time in CRU post infusion will be the clinical decision of the Investigator. After Week 28, if appropriate in the opinion of the Investigator, home health care visits may be utilized. Patients will be required to return to the CRU every 24 weeks starting at Week 96, or as clinically indicated in the opinion of the Investigator.
[o] Vital signs will be obtained within 60 min prior to dosing (for either IV or SC dosing) starting at Week 97.

Table 7: Schedule of Assessments for Year 4 (Weeks 145 to 192)

	Weekly (QW) Dosing											
	Wk 148	**Wk 152**	**Wk 156**	**Wk 160**	**Wk 164**	**Wk 168**	**Wk 172**	**Wk 176**	**Wk 180**	**Wk 184**	**Wk 188**	**Wk 192**
Height[a]						X						X
Weight[b]			X			X			X			X
Physical examination						X						X
ECG												
Clinical laboratory tests						X						X
Pregnancy test[c]	X	X	X	X	X	X	X	X	X	X	X	X
3-day diet record[d]												
Pegzilarginase dosing[e]	Weekly											
Arginine/ornithine[f]						X						X
Guanidino compounds[f]						X						X
ADA[g]												
Other amino acids (glu, val, leu, ile)[g]												
Neurological/neuromotor, adaptive behavior, and QoL assessments[h]												
Neurocognitive and developmental assessments[i]												
Clinical growth assessments[j]						X						X
Clinic/CRU visit[k]						X						X
Vital signs[l]	Weekly											
Concomitant medications	Weekly											
Adverse events	Weekly											
Seizure frequency	Weekly											

ADA = anti-drug antibodies (anti-pegzilarginase antibodies); ALT = alanine aminotransferase; AST = aspartate aminotransferase; CRU = clinical research unit; ECG = electrocardiogram; EOT/SFU = end of treatment/study follow-up visit; glu = glutamine; ile = isoleucine; IV = intravenous; leu = leucine; PEG = polyethylene glycol; PK = pharmacokinetic; SC = subcutaneous; QOL = quality of life; QW = once weekly; val = valine; Wk = week.

[a] Height will be collected every 24 weeks for pediatric patients only..

[b] Weight will be obtained during the baseline period and approximately every 12 weeks during dosing starting at Week 96. The baseline weight can be used to calculate each total dose, unless a subsequent weight obtained indicates a > 10% change from the baseline weight.

[c] Pregnancy test can either be a serum or a urine test.

[d] The 3-day diet record will not be required in after Week 144 other than at the EOT/SFU visit.

[e] See Section 9.7.1 for details regarding dosing, including frequency and route.
[f] Samples for arginine, ornithine, and guanidino compounds will be collected prior to dosing every 24 weeks or as clinically indicated starting at Week 144. Samples will be collected via venipuncture or via fingerstick or via alternate methods as outlined in the lab manual once they are available and will be sent to a designated central laboratory. An approximate 2-hour fast should be observed prior to all sampling for amino acids and within 1 hour prior to dose, except where operationally and/or clinically not possible.
[g] ADAs and other amino acid samples will not be collected after Week 144 other than at the EOT/SFU visit.
[h] Neurological/neuromotor, adaptive behavior, and QoL assessments will not be collected after Week 144 other than at the EOT/SFU visit.
[i] Neurocognitive and developmental assessments will not be collected after Week 144 other than at the EOT/SFU visit.
[j] Growth assessments (age-appropriate length/height, weight, head circumference measures) will be performed for pediatric patients (ages 2 to 18) every 24 weeks and at the EOT/SFU visit:
[k] Admission to CRU and observation time in CRU post infusion will be the clinical decision of the Investigator. After Week 28, if appropriate in the opinion of the Investigator, home health care visits may be utilized. Patients will be required to return to the CRU every 24 weeks starting at Week 96, or as clinically indicated in the opinion of the Investigator.
[l] Vital signs will be obtained within 60 min prior to dosing (for either IV or SC dosing) starting at Week 97.

Table 8: Schedule of Assessments Post Week 192

	Post Week 192 Study Visits				
Assessments	**Q week**	**Q4 weeks**	**Q12 weeks**	**Q24 weeks**	**Safety[m] Follow up/EOT**
Height[a]				X	X
Weight[b]			X		X
Physical examination				X	X
ECG					X
Clinical Laboratory Tests				X	X
Pregnancy Test[c]		X			X
3-day diet record[d]					X
Arginine/ornithine[f]				X	X
Guanidino compounds[f]				X	X
ADA[g]					X
Other amino acids (glu, val, leu, ile)[g]					X
Neurological/neuromotor, adaptive behavior, and QoL assessments[h]					X
Neurocognitive and developmental assessments[i]					X
Clinical growth assessments[j]				X	X
Vital Signs[l]				X	X
Adverse Events	X				X
Concomitant medications	X				X

Seizure frequency	X				X
Pegzilarginase dosing [e]	X				
Clinic/CRU visit[k]				X	X

ADA = anti-drug antibodies (anti-pegzilarginase antibodies); ALT = alanine aminotransferase; AST = aspartate aminotransferase; CRU = clinical research unit; ECG = electrocardiogram; EOT/SFU = end of treatment/study follow-up visit; glu = glutamine; ile = isoleucine; IV = intravenous; leu = leucine; PEG = polyethylene glycol; PK = pharmacokinetic; SC = subcutaneous; QOL = quality of life; QW = once weekly; val = valine; Wk = week.

[a] Height will be collected every 24 weeks for pediatric patients only.

[b] Weight will be obtained during the baseline period and approximately every 12 weeks during dosing starting at Week 96. The baseline weight can be used to calculate each total dose, unless a subsequent weight obtained indicates a > 10% change from the baseline weight.

[c] Pregnancy test can either be a serum or a urine test.

[d] The 3-day diet record will not be required after Week 144 other than at the EOT/SFU visit.

[e] See Section 9.7.1 for details regarding dosing, including frequency and route.

[f] Samples for arginine, ornithine, and guanidino compounds will be collected prior to dosing every 24 weeks or as clinically indicated starting at Week 144. Samples will be collected via venipuncture or via fingerstick or via alternate methods as outlined in the lab manual once they are available and will be sent to a designated central laboratory. An approximate 2-hour fast should be observed prior to all sampling for amino acids and within 1 hour prior to dose, except where operationally and/or clinically not possible.

[g] ADAs and other amino acid samples will not be collected after Week 144 of the study other than at the EOT/SFU visit.

[h] Neurological/neuromotor, adaptive behavior, and QoL assessments will not be collected after Week 144 of the study other than at the EOT/SFU visit

[i] Neurocognitive and developmental assessments will not be collected after Week 144 of the study other than at the EOT/SFU visit

[j] Growth assessments (age-appropriate length/height, weight, head circumference measures) will be performed for pediatric patients (ages 2 to 18) every 24 weeks and at the EOT/SFU visit.

[k] Admission to CRU and observation time in CRU post infusion will be the clinical decision of the Investigator. After Week 28, if appropriate in the opinion of the Investigator, home health care visits may be utilized. Patients will be required to return to the CRU every 24 weeks starting at Week 96, or as clinically indicated in the opinion of the Investigator.

[l] Vital signs will be obtained within 60 min prior to dosing every 24 weeks starting at Week 193.

[m] Safety Follow-up visit will be conducted approximately 2 weeks after last dose of pegzilarginase.

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4. LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Definition
ADA	anti-drug antibodies (anti-pegzilarginase antibodies)
ADHD	attention-deficit/hyperactivity disorder
AE	adverse event
AL	argininosuccinic acid lyase
ALT	alanine aminotransferase
aPTT	activated partial thromboplastin time
AS	argininosuccinic acid synthase
AST	aspartate aminotransferase
ATS	American Thoracic Society
$AUC_{0-\infty}$	area under the concentration versus time curve from time zero to infinity
AUC_{0-168}	area under the concentration versus time curve from time zero across the dosing interval (168 hours or 1 week)
BBS	Berg Balance Scale
BSID-III	Bayley Scales of Infant and Toddler Development III
BUN	blood urea nitrogen
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
CITR	aspartate transporter
Co-Arg1-PEG	Pegzilarginase drug substance
CPSI	carbamoylphosphate synthase
CRA	clinical research associate
CRO	contract research organization
CRU	clinical research unit
DSMB	Data Safety Monitoring Board
EC	Ethics Committee
ECG	electrocardiogram
eCRF	electronic case report form
EDC	electronic data capture
EOI	end of infusion
EOT	end of treatment
F	bioavailability
FAS	Full Analysis Set
FDA	Food and Drug Administration
GAI	General Ability Index
GC	guanidino compounds

Abbreviation	**Definition**
GCP	Good Clinical Practice
GI	gastrointestinal
GLP	Good Laboratory Practice
Glu	glutamine
GMFM	Gross Motor Function Measure
HRQoL	health-related quality of life
HIPAA	Health Insurance Portability and Accountability Act
HIV	human immunodeficiency virus
IB	Investigator's Brochure
ICF	informed consent form
ICH	International Council for Harmonisation
Ile	isoleucine
INR	international normalized ratio
IP	investigational product: "A pharmaceutical form of an active ingredient or placebo being tested or used as a reference in a clinical trial, including a product with a marketing authorization when used or assembled (formulated or packaged) in a way different from the approved form, or when used for an unapproved indication, or when used to gain further information about an approved use." (from ICH E6). The terms "IP" and "study drug" may be used interchangeably in the protocol.
IQ	intelligence quotient
IRB	Institutional Review Board
ITT	intent-to-treat
IUD	intrauterine device
IUS	intrauterine hormone-releasing system
IV	intravenous
KABC-II	Atlantis Subtest on the Kaufman Assessment Battery for Children-II
MAS	Modified Ashworth Scale
MedDRA	Medical Dictionary for Regulatory Activities
MTD	maximum tolerated dose
NAGS	N-acetylglutamate synthase
NOAEL	no observed adverse effect level
NOS	nitric oxide synthase
ORNT	ornithine transporter
OTC	ornithine transcarbamoylase
PROMIS	Patient-Reported Outcomes Measurement Information System
PD	pharmacodynamic
PedsQL	Pediatrics Quality of Life

Abbreviation	**Definition**
PEG	polyethylene glycol
PK	pharmacokinetic(s)
PT	partial thromboplastin
QoL	quality of life
QW	once weekly
RAVLT	Rey Auditory Verbal Learning Test
RBC	red blood cell
ROM	range of motion
SAE	serious adverse event
SAP	statistical analysis plan
SC	subcutaneous
SFU	study follow-up
6MWT	Six-Minute Walk Test
TOVA	Test of Variables of Attention
ULN	upper limit of normal
US	United States
Val	valine
WAIS-IV	Wechsler Adult Intelligence Scale IV
WBC	white blood cell
WISC-V	Wechsler Intelligence Scale for Children V
Wk	week
WPPSI-IV	Wechsler Preschool and Primary Scale of Intelligence IV
ZBI-12	Short Form Zarit Burden Interview

5. ETHICS

5.1. Institutional Review Board or Ethics Committee

Prior to initiating the study, the Investigator will obtain written confirmation that the Institutional Review Board (IRB) or Ethics Committee (EC) is properly constituted and compliant with International Council for Harmonisation (ICH) Guidelines and good clinical practice (GCP) requirements, applicable laws and local regulations. A copy of the confirmation from the IRB/EC will be provided to Aeglea BioTherapeutics or designee. The principal investigator will provide the IRB/EC with all appropriate material, including the protocol, Investigator's Brochure (IB), the informed consent form (ICF) including compensation procedures, and any other written information provided to the patients, including all consent forms translated to a language other than the native language of the clinical site. The study will not be initiated and investigational product (IP) supplies will not be shipped to the site until appropriate documents from the IRB/EC confirming unconditional approval of the protocol, the ICF, and all patient recruitment materials are obtained in writing by the Investigator and copies are received at Aeglea or designee. The approval document should refer to the study by protocol title and Aeglea Study Number (if possible), identify the documents reviewed, and include the date of the review and approval. Aeglea or designee will ensure that the appropriate reports on the progress of the study were made to the IRB/EC and Aeglea by the Investigator in accordance with applicable governmental regulations.

5.2. Ethical Conduct of Study

This study will be conducted in accordance with the following:

1. United States (US) Code of Federal Regulations (CFR) sections that address clinical research studies, and/or other national and local regulations, as applicable
2. E6 ICH Guideline for Good Clinical Practice

Specifically, this study is based on adequately performed laboratory and animal experimentation; the study will be conducted under a protocol reviewed and approved by an IRB/EC; the study will be conducted by scientifically and medically qualified persons; the benefits of the study are in proportion to the risks; the rights and welfare of the patients will be respected; the physicians conducting the study do not find the hazards to outweigh the potential benefits; and each patient, or his/her legally authorized representative, will provide written, informed consent before any study–related tests or evaluations are performed.

5.3. Patient Information and Informed Consent

A properly written and executed ICF, in compliance with ICH E6 (GCP Guideline, Section 4.8) and 21 CFR §50 and other applicable local regulations, will be obtained for each patient or their caregiver prior to the conduct of any study-specific procedures. If the patient or caregiver is unable to read, an impartial witness should be present during the entire informed consent discussion. The IRB/EC must approve the documents before they are implemented. A copy of the approved ICF, and if applicable, a copy of the approved patient information sheet, and all ICFs translated to a language other than the native language of the clinical site, must also be received by Aeglea or designee prior to the delivery of IP.

The Investigator will provide copies of the signed ICF to each patient and will maintain the original in the patient's file.

6. INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

Prior to enrolling patients into the study, the Investigator at each site must provide to Aeglea BioTherapeutics, Inc. or designee a fully executed and signed Form FDA 1572 and a Financial Disclosure Form for the Investigator. All sub–Investigators must be listed on Form FDA 1572. Financial Disclosure Forms must also be completed for all sub–Investigators listed on the Form FDA 1572 who will be directly involved in the treatment or evaluation of research patients in this study.

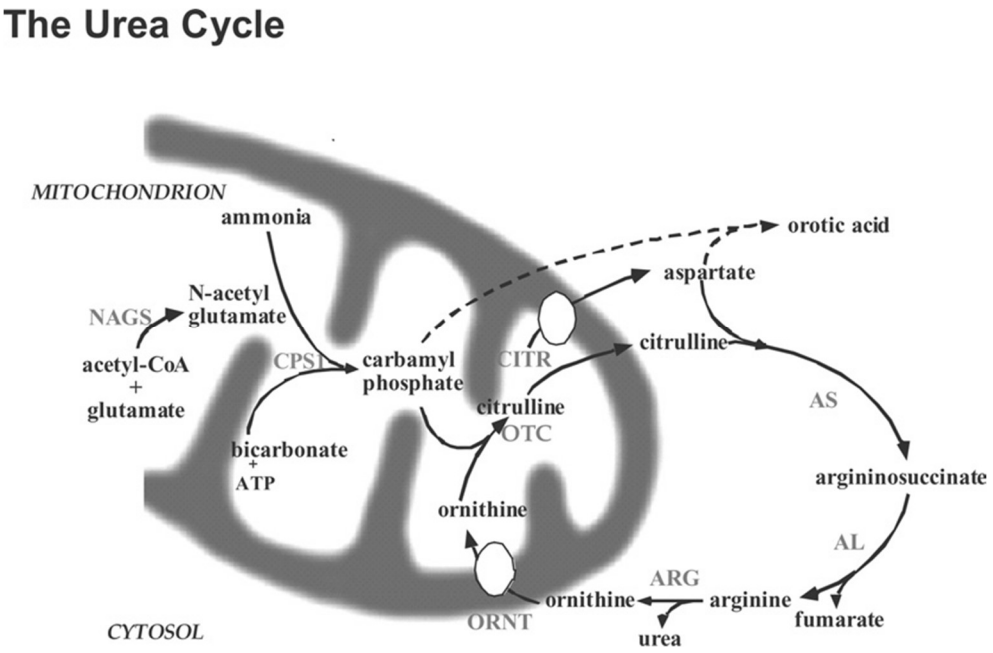
The study will be administered and monitored by employees or representatives of Aeglea or designee. Clinical Research Associates (CRAs) or trained designees will monitor each site on a periodic basis and perform verification of source documentation as well as other required review processes. Aeglea Regulatory Affairs Department or designee will be responsible for the timely reporting of serious adverse events (SAEs) to appropriate regulatory authorities as required.

7. INTRODUCTION

7.1. Overview of Arginase 1 Deficiency

Arginase 1 deficiency or hyperargininemia (Orpha number ORPHA90; ICD-10 code: E72.2; OMIM number 207800) is a rare disorder of amino acid metabolism caused by a deficiency in the enzyme Arginase 1 [ARG1 (EC 3.5.3.1)]. Arginase 1 is one of the 6 enzymes critical to the normal function of the urea cycle Figure 1:); it catalyzes the conversion of L-arginine to urea and ornithine in the last step of the cycle. The ornithine then reenters the mitochondrion to continue the cycle (Cederbaum 2012; Feldman 2010).

Figure 1: The Urea Cycle



The Urea Cycle. The 6 primary enzymatic steps of the urea cycle are shown in bold capital letters. NAGS, N-acetylglutamate synthase; CPSI, carbamoylphosphate synthase; OTC, ornithine transcarbamoylase; AS, argininosuccinic acid synthase; AL, argininosuccinic acid lyase; and ARG, arginase. ORNT and CITR are respectively, the ornithine and aspartate transporters. Their function is necessary for the movement of substrates in and out of the mitochondrion (Crombez 2005).

Arginase 1 is found predominantly in red blood cells (RBCs) and the liver, while Arginase II is found predominantly in the kidney and prostate. ARG1 is the only gene in which mutations are known to cause Arginase 1 deficiency; no human deficiency state has been identified for Arginase II. Arginase deficiency is inherited in an autosomal recessive manner and has an estimated incidence of 1:950,000 live births (Schlune 2015).

Clinically, Arginase 1 deficiency is characterized by a slow deterioration of the cerebral cortex and pyramidal tracts that leads to progressive dementia, psychomotor retardation, spastic diplegia, seizures, and growth failure. Generally, birth and early infancy are relatively normal, but linear growth slows between 1 and 3 years of age and is followed by the development of spasticity, plateauing of cognitive development, and loss of developmental milestones. Given the similarity of many of the neurological manifestations of hyperargininemia to those of cerebral palsy, the diagnosis of this disorder has often been delayed, resulting in patients with significant

neurocognitive impairment. In recent years, neonatal testing for arginase deficiency has been available in approximately 34 states in the US, allowing for earlier intervention in some patients (Therrell 2017). If left untreated, however, the disease progresses to severe spasticity, loss of ambulation, loss of bowel and bladder control, and severe intellectual disability. Patients with Arginase 1 deficiency typically have elevated blood arginine levels (3 to 4 times the upper limit of normal [ULN]), mild hyperammonemia, and a mild increase in urinary orotic acid. Most patients have no detectable Arginase 1 enzyme activity in RBCs (<1% of normal) (Cederbaum 2012; Feldman 2010). Hyperammonemia is less common in Arginase 1 deficiency than the other urea cycle disorders, but hyperammonemic coma and death have been reported. The hyperammonemia seen with hyperargininemia generally responds readily to conservative management such as fluid replacement, nitrogen scavenging agents, and treatment of the inciting illness.

The current treatment of Arginase 1 deficiency is focused on the maintenance of plasma arginine concentrations at a level as near to normal as possible through lifelong dietary protein restriction. Protein intake is limited to the minimum required to maintain protein biosynthesis and growth. Half or more of dietary protein is given in the form of an arginine-free essential amino acid mixture. Such dietary modification can reduce plasma arginine levels in most patients, but the diet is unpalatable, expensive, and difficult to maintain and manage especially in growing children. Therapy with glycerol phenylbutyrate or sodium benzoate is used to reduce elevated ammonia levels. Liver transplantation has been reported to achieve disease normalization in some patients, but despite these successes, this intervention is available to only a small fraction of patients and carries a significant risk of mortality and morbidity.

The paucity of treatment options for patients deficient in Arginase 1 highlights the significant unmet need for a therapy that will lower arginine levels to within the normal range and promote the lifelong maintenance of normal arginine levels. The development of such a therapeutic could be useful in the attempt to minimize exposure to the neurotoxic effects of arginine and its metabolites and offer the potential for normal neurocognitive development in these patients.

7.2. Pegzilarginase

Pegzilarginase (Co-Arg1-PEG, AEB1102) is a cobalt-substituted, pegylated human recombinant arginase 1 enzyme expressed in *E. coli* that is formulated as a solution for intravenous (IV) or subcutaneous (SC) injection. The substitution of the native manganese (Mn^{2+}) with cobalt (Co^{2+}) in the active site of arginase 1 enhances the stability and catalytic activity at physiological pH. Pegylation extends the circulating half-life. Please refer to the latest version of the IB for additional details of the development of pegzilarginase and nonclinical studies.

Given the characteristics of pegzilarginase and the relatively low doses required to reduce arginine levels into the normal range, both IV and SC dosage forms have been developed. Subcutaneous delivery may be a more convenient method of administration and is expected to facilitate home administration.

In this study, patients with Arginase 1 deficiency may receive pegzilarginase by both the IV and SC routes. This is the first clinical study where pegzilarginase will be administered subcutaneously.

7.3. Other Clinical Studies with Pegzilarginase

Please refer to the latest version of the IB for additional details of the prior clinical experience with pegzilarginase.

7.4. Subcutaneous (SC) Dosing of Pegzilarginase

A Good Laboratory Practice (GLP) toxicology study in monkeys with once weekly (QW) SC administration of pegzilarginase for 4 weeks was completed (Study 2461-008/AEB-002-1152). The highest SC dose of 0.5 mg/kg was the no observed adverse effect level (NOAEL) in this study. The bioavailability (F) could not be estimated in this study; therefore, the bioavailability was determined to be ~62% at 0.5 mg/kg from an earlier non-GLP, single dose IV/SC monkey pharmacokinetics (PK) study (Study 2461-007/AEB-002-1102) using a similar SC formulation. The estimated safety margins based on 62% bioavailability provide mean predicted SC exposures in patients ranging from 62-fold at 0.015 mg/kg pegzilarginase to 7.9-fold at 0.06 mg/kg Table 9. If 100% bioavailability is assumed, the margins are reduced to ~4.9-fold at the 0.06 mg/kg dose, ~10-fold at the 0.04 mg/kg dose, and ~39-fold at the 0.015 mg/kg dose.

Please refer to the latest version of the IB for additional details on the SC route of administration.

Table 9: Safety Margins for SC Doses of Pegzilarginase at 0.015 and 0.06 mg/kg in Patients with Arginase 1 Deficiency

Preclinical Data (Day 22)			**Human Data (Day 1)**			
Species (GLP Study No.)	**Dose (mg/kg)**	**$AUC_{0\text{-}168}$ (hr•µg/mL)**	**Dose (mg/kg)**	**Observed IV $AUC_{0\text{-}\infty}$ (hr•µg/mL)**	**Predicted SC $AUC_{0\text{-}\infty}$ (hr•µg/mL)[a]**	**Safety Margin[b]**
Monkey (AEB-002-1152)	0.5 (NOAEL)	405 ± 33.4[c]	0.015	10.5	6.51	~62
			0.04	39.9	24.7	~16
			0.06	82.2	51.0	~7.9

$AUC_{0\text{-}\infty}$ = area under the concentration versus time curve from time zero to infinity; $AUC_{0\text{-}168}$ = area under the concentration versus time curve from time zero across the dosing interval (168 hr or 1 week); F = bioavailability; GLP = Good Laboratory Practices; IV = intravenous; NOAEL = no observed adverse effect level; SC = subcutaneous

[a] Predicted SC $AUC_{0\text{-}\infty} = F \times AUC_{0\text{-}\infty,IV} = 0.62 \times AUC_{0\text{-}\infty,IV}$;

[b] The safety margin(s) were calculated as mean $AUC_{0\text{-}168,monkey} / AUC_{Human}$.

[c] Data shown are mean ± standard deviation.

7.5. Summary of Potential Risks and Benefits

The potential risks of pegzilarginase administration are presented below and are based on data from dose range-finding and GLP toxicology studies performed to date, on adverse events (AEs) observed in clinical studies with pegzilarginase, and from information available in the medical literature.

7.5.1. Potential Risks

Please refer to the latest version of the IB for additional details of the prior clinical experience with pegzilarginase.

7.5.1.1. Hypoargininemia

Patients with Arginase 1 deficiency generally have plasma arginine levels well above the age-adjusted normal range, even when compliant with a protein restricted diet. From the Framingham Offspring Study the reference range for arginine was determined to be 41 – 114 µmol/L (Lüneberg 2011). The treatment goal with pegzilarginase in ARG1-D patients, is to dose pegzilarginase at a level to reduce plasma arginine levels as recommended by the medical guideline (Häberle 2012) with optimum time within normal range.

It is possible that pegzilarginase activity may cause transient plasma arginine levels below < 40 µmol/L in patients with ARG1-D; however, the likelihood of prolonged arginine depletion is low and the risk of adverse event related to plasma arginine < 40 µmol/L is unlikely.

Arginine deficiency syndromes appear to primarily affect endothelial or immune cell function and specific clinical consequences of arginine deficiency are dependent on the pathophysiologic context in which the deficiency occurs (Morris 2012). Rapid normalization of plasma arginine in Arginase 1 deficiency patients has been observed clinically after liver transplantation with no apparent physiologic effects on these patients (Santos Silva 2013).

This clinical study will include patients who maintain a protein-restricted diet and have elevated blood arginine concentrations. Following administration of pegzilarginase, study patients will be monitored, with blood arginine determinations. Dose modifications may occur during the study as clinically indicated based on PD response.

7.5.1.2. Immunogenicity

Pegzilarginase is derived from native human Arginase 1, with modifications only in the enzyme cofactor (Mn^{+2} to Co^{+2}) and in the pegylation used to enhance its stability. It is expected that pegylation will mask some epitopes that could play a role in the immune response to pegzilarginase. Nonetheless, the differences between pegzilarginase and the native human enzyme may produce an immune response in patients; patients whose genetic defect results in a total absence of the Arginase 1 protein may be particularly susceptible.

Patients participating in this clinical trial of pegzilarginase will be monitored for signs and symptoms of hypersensitivity or anaphylaxis, and for the development of anti-drug antibodies (ADAs) as well as loss of enzymatic activity. During the course of the trial, patients will remain on their previously prescribed dietary protein restriction as clinically indicated to avoid acute negative consequences associated with such a loss of arginase activity. Should neutralizing ADAs develop affecting safety, efficacy, and/or enzyme activity, consideration will be given to the development of a tolerizing treatment regimen. Patients will also be monitored for the development of anti-polyethylene glycol (PEG) antibodies and any loss of activity related to such antibodies. Patients will be monitored for signs and symptoms associated with potential immune reactions to the administration of pegzilarginase.

7.5.1.3. Toxicity Related to Polyethylene Glycol (PEG) Metabolites

The risk of significant exposure to PEG metabolites including ethylene glycol and diethylene glycol following treatment with pegylated proteins is considered to be relatively low.

Calculations of systemic exposure to these metabolites following administration of a dose of pegzilarginase, assuming that all of the administered PEG is converted to ethylene glycol, indicates that doses < 1.0 mg/kg pegzilarginase in humans may result in highest possible ethylene glycol exposures of approximately 14 mg/day. However, recognizing that the likelihood of complete conversion of PEG to ethylene or diethylene glycol is low, the risk of overexposure to these compounds in pegzilarginase clinical trials is considered to be minimal.

7.5.1.4. Hematologic Adverse Effects

Hematologic effects observed in nonclinical toxicology studies included a reversible decrease in RBC mass, moderate to marked decreases in neutrophil counts, and a reversible decrease in lymphocyte counts.

7.5.1.5. Gastrointestinal Adverse Effects

Gastrointestinal (GI) adverse effects, including decreased appetite, vomiting, and diarrhea were observed in the nonclinical toxicology studies. Patients participating in this clinical study of pegzilarginase will be monitored for GI adverse effects.

7.5.1.6. Cardiovascular Effects

Modest increases in heart rate, within historical controls for the test facility, were observed in monkeys treated at the highest dose level of pegzilarginase.

Arginine is a substrate for both arginase and nitric oxide synthase (NOS). In theory, high levels of arginase may compete with NOS for arginine resulting in decreased nitric oxide levels, a reduced ability to decrease vascular smooth muscle cell relaxation, leading to increases in blood pressure (Durante 2007). Patients with low endothelial NOS could therefore exhibit heightened sensitivity to arginine depletion. In the advanced solid tumor study, 1 subject at the 0.18 mg/kg dose experienced transient, asymptomatic Grade 3 hypertension at approximately 9 hours after the initiation of treatment (Cycle 1, Day 1). The event did not require therapeutic intervention and was self-limiting within 4 hours. The Investigator considered the event probably related to study drug.

Vital signs, including heart rate and blood pressure, will be monitored in patients receiving pegzilarginase. Electrocardiograms (ECGs) will also be obtained to assess potential cardiovascular effects associated with pegzilarginase administration. Study drug may held in patients experiencing a severe or life-threatening AE considered likely to be related to pegzilarginase. Dosing may be halted in any patient experiencing a severe or life-threatening adverse effect (Section 9.8).

7.5.1.7. Reproductive Effects

No reproductive effects were observed in the non-clinical toxicology studies in mice and monkeys that were conducted in young adult animals. In contrast, mild degeneration/atrophy of the seminiferous tubules, decreased sperm in the epididymides, reduced sperm motility,

increased percentage of abnormal sperm, and a decrease in size of the prostate, coagulating glands and seminal vesicles were observed in male juvenile rats (treatment initiated at 3 weeks of age) treated at 1 mg/kg of pegzilarginase QW for 13 weeks. No reproductive effects were observed in female juvenile rats. The initial studies of pegzilarginase will be conducted in adult patients with advanced solid tumors; adults are not expected to experience this effect. The initial studies of pegzilarginase in patients with Arginase 1 deficiency will include pediatric and adult patients. It is expected that exposure to pegzilarginase in human clinical trials will be lower than the exposure associated with reproductive adverse effects in male rats. Nonetheless, male patients, particularly peri-pubescent patients, will be monitored carefully for any adverse effects related to the reproductive system, with attention to clinical symptoms or physical examination findings, and urological referral and evaluation if needed. The ICF will specifically note this potential adverse effect. Additionally, highly effective methods of contraception will be required for sexually active male and female patients throughout participation in the study. Highly effective methods of contraception include: combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation; progesterone-only hormonal contraception associated with inhibition of ovulation; intrauterine device (IUD); or intrauterine hormone-releasing system (IUS).

7.5.2. Potential Benefits

Despite treatment with a protein-restricted diet, patients with Arginase 1 deficiency may have plasma levels of arginine greater than 3 times higher than the normal range (Huemer 2016). Observations from case reports which describe a small number of patients who have achieved reasonable improvements in plasma arginine levels with strict dietary control provide important orthogonal data supporting the hypothesis that lowering arginine can improve in one or more clinical parameters, including spasticity, mobility, muscle strength, swallowing, cognitive function, or speech (Cederbaum 1979, Cederbaum 1982, Lambert 1991, Prasad 1997). Despite these improvements, arginine levels are rarely and inconsistently normalized, and improvement in clinical parameters is incomplete. Based on this information, it is unlikely that improvement in clinical outcomes will be observed without marked and sustained reduction in plasma arginine. Pegzilarginase has been shown to reduce arginine levels in patients with hyperargininemia and in patients with solid tumors and hematologic tumors. It is expected that treatment with pegzilarginase will reduce arginine levels in patients with Arginase 1 deficiency and thus may alleviate some of the symptoms and signs of this disease. It is also hypothesized that treatment may slow or halt the progression of neurological and neurocognitive deterioration in these patients. These potential benefits are considered to outweigh the potential risks described above and support the clinical investigation of pegzilarginase for the treatment of Arginase 1 deficiency.

8. STUDY OBJECTIVES

8.1. Primary Objective

- To evaluate the long-term safety and tolerability of IV or SC pegzilarginase administered in patients with Arginase 1 deficiency and hyperargininemia

8.2. Secondary Objectives

- To evaluate the long-term effects of pegzilarginase in patients with Arginase 1 deficiency and hyperargininemia on sustained reduction of arginine levels
- To evaluate the long-term effects of pegzilarginase on plasma guanidino compounds
- To characterize repeat-dose PK of pegzilarginase administered IV or SC
- To evaluate the long-term effects of pegzilarginase on stabilization or improvement of neurological/neuromotor manifestations, using objective measures:
  - Six-Minute Walk Test (6MWT)
  - Berg Balance Scale (BBS)
  - Gross Motor Function Measure (GMFM)
  - Modified Ashworth Scale (MAS)
  - Purdue Pegboard Test
- To evaluate the long-term effects of pegzilarginase on select manifestations of Arginase 1 deficiency:
  - Frequency of seizures
  - Frequency of hyperammonemic episodes
  - Incidence of disease-related transaminase elevations

8.3. Exploratory Objectives

- To compare the PK and PD of different routes (IV and SC) of pegzilarginase administration
- To examine the long-term changes in exploratory biomarkers including additional amino acids (ornithine, glutamine, leucine, isoleucine, and valine) and blood urea nitrogen (BUN) after administration of pegzilarginase
- To evaluate the long-term effects of pegzilarginase on stabilization or improvement of neurocognitive, developmental, and quality of life (QoL) measures, selected based on patient age:
  - Intelligence:
    a. Wechsler Adult Intelligence Scale IV (WAIS-IV)
    b. Wechsler Intelligence Scale for Children V (WISC-V)
    c. Wechsler Preschool and Primary Scale of Intelligence IV

d. Bayley Scales of Infant Development III

– Memory:

a. Rey Auditory Verbal Learning Test
b. Atlantis subtest on the Kaufman Assessment Battery for Children II

– Attention:

a. Test of Variables of Attention (TOVA) version 9

– Adaptive Behavior:

a. Vineland Adaptive Behavior Scales II

– Development:

a. Growth parameters (length/stature, weight, head circumference)

– QoL:

a. Patient-Reported Outcomes Measurement Information System (PROMIS) or Pediatric Quality of Life Inventory (PedsQL)
b. Short Form Zarit Burden Interview (ZBI-12)

9. INVESTIGATIONAL PLAN

9.1. Overall Study Design and Plan

This is a long-term, open-label extension study of pegzilarginase in approximately 10 pediatric and adult patients with Arginase 1 deficiency and hyperargininemia who participated in Study 101A. The Investigator and the Sponsor will evaluate each individual patient's safety information available at the time of Study 101A completion to determine if it is acceptable for the patient to be enrolled in this study. Patients must have tolerated the single and repeat-doses of pegzilarginase without experiencing any clinically significant event that would preclude continued dosing and must continue to meet applicable eligibility criteria from the original study.

Dosing in an individual patient will commence approximately 4 weeks (but no sooner than 3 weeks) after the last dose in Study 101A. The pegzilarginase dose level and frequency will initially match the dose and regimen the patient was receiving at the end of Study 101A but may be adjusted if clinically indicated in the opinion of the Investigator in consultation with the Sponsor. During the course of the study, doses and route of administration may be adjusted for an individual based on available PK/PD data or for safety and/or tolerability reasons as clinically indicated in the opinion of the Investigator in consultation with the Sponsor. Changes to route of administration should be avoided during the first 24 weeks of the study.

The first 24 doses in this study will be administered using the IV route weekly. The first 12 doses in this study should be administered IV weekly in the clinical research unit (CRU). After the initial 12 IV doses, patients may be dosed outside of the CRU (ie, by home health care professionals) if considered safe and appropriate in the opinion of the Investigator in consultation with the Sponsor.

The recommended dosing schedule is as follows:

- IV administration QW for Weeks 1-24
- SC administration weekly for Weeks 25-48
- As clinically indicated thereafter until study termination(Investigator decision in consultation with the Sponsor)

Safety, tolerability, immunogenicity, PK, and PD assessments as evaluated in Study 101A will continue for the duration of this long-term extension study, although with different frequency. The Data Safety Monitoring Board (DSMB) will periodically provide independent review of the safety, tolerability, immunogenicity, PK, and PD measures during the study.

A follow-up visit will be scheduled approximately 2 weeks after the completion of the final dose of pegzilarginase; at this visit, the patient's participation in the study will end.

9.2. Impact of Coronavirus Disease (COVID-19)

The COVID-19 pandemic has impacted clinical research. The safety of study participants is paramount and must be at the heart of every decision, regardless of any potential consequences for an ongoing study. Consequently, various regulatory authorities have issued guidance on how to manage the impact of this pandemic on the methodological aspects of ongoing research (FDA September 2020, EMA April 2020, EMA June 2020).

There is an ethical mandate to proceed with a study that has been started so that the efforts taken by study participants and physicians can benefit drug development and inform patient care. Guidance strongly recommends that sponsors integrate all available knowledge from the ethical, medical, and methodological perspective into decision making about the future conduct of a study while carefully considering advice from regulatory and healthcare authorities. General advice on how the different aspects related to the COVID-19 pandemic should be handled is not yet feasible, as the implications on clinical studies are expected to be manifold. COVID-19's impact on recruitment, data collection, and analysis and interpretation of results for each study needs a thorough case-by-case assessment.

Due to the evolving nature of the COVID-19 pandemic and the variation in guidance and restrictions across countries and sites, an amendment for specific anticipated deviations is not feasible. Investigators as well as study sites and regulatory bodies such as IRBs/ECs are encouraged to adapt study procedures to ensure subject safety and compliance with local and regulatory procedures within the context of this protocol. Any variance from this study's assessments and procedures should be clearly documented and captured as protocol deviations and annotated as a result of the COVID-19 pandemic.

9.3. Number of Patients

All qualified pediatric and adult patients who participated in the 101A study will be allowed to participate in this study.

9.4. Expected Duration of Patient Participation

Each patient is expected to receive pegzilarginase in this study following the completion of Study 101A until pegzilarginase is available through other means.

9.5. Discussion of Study Design, Including Choice of Control Group

This will be an open-label, long-term extension study designed to evaluate the safety, tolerability, and efficacy of administration of pegzilarginase. On-treatment arginine levels will be compared with arginine levels determined prior to treatment.

Safety, tolerability, and efficacy of pegzilarginase will be evaluated by assessment of AEs, physical examinations, vital signs, ECGs, clinical laboratory parameters, ammonia levels, and amino acid levels. The PK profile and immunogenicity of pegzilarginase will be evaluated through serial blood sampling.

The SC dosing route is being investigated in this study as an option when considered safe by the investigator given the potential to address patient and parent/caregiver burden.

The provisions for home health care dose administration and assessments and SC dosing route have been included to alleviate patient and parent/caregiver burden and to evaluate the use of pegzilarginase outside a clinic setting. The provisions for home health care dose administration may be utilized for some patients after minimum mandated dosing periods have been completed and where considered safe and appropriate by the investigator in consultation with the sponsor.

Blood samples will also be collected to evaluate (in an exploratory manner) the exposure of patients to guanidino compounds (GC), which are direct and indirect products of arginine

metabolism; many GCs are elevated in Arginase 1 deficiency. These compounds may contribute to the pathophysiological changes in Arginase 1 deficiency, such as seizures and hepatic dysfunction, though exact causal relationships and mechanisms are not well understood.

9.6. Selection of Study Population

9.6.1. Inclusion Criteria

A patient must meet all of the following criteria to be enrolled in this study:

1. Completed participation in Study CAEB1102-101A without experiencing any clinically significant AE or other unmanageable drug toxicity that precludes continued dosing
2. After review of the patient's data from the CAEB1102-101A study, the Investigator and the Sponsor confirm that it is acceptable for the patient to continue dosing with AEB1102 and that the patient continues to meet the following criteria from the 101A study:
    a. Is male or female: pediatric patients ≥2 to <18 years of age and adult patients ≥18 years of age
    b. Has documented diagnosis of Arginase 1 deficiency, with:
        i. Known mutation in the Arginase 1 gene

OR

        ii. Known deficiency in RBCs arginase activity

    c. Has adequate organ function defined as follows:
        i. Bone Marrow: Hemoglobin ≥10 g/dL; absolute neutrophil count ≥1.5 x10^9/L; platelet count ≥ 100,000/μL
        ii. Hepatic: transaminase levels (aspartate aminotransferase/alanine aminotransferase ≤2.5-times ULN; total bilirubin ≤ 2.0 mg/dL
        iii. Renal: serum creatinine < 1.5 x ULN
3. If female and of child-bearing potential, has a negative serum pregnancy test within 7 days before enrollment
4. If the patient (male or female) is engaging in sexual activity that could lead to pregnancy, must be surgically sterile, post-menopausal (female), or must agree to use a highly effective method of birth control during the study and for a minimum of 30 days after the last study drug administration. Highly effective methods of contraception include: combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation; progesterone-only hormonal contraception associated with inhibition of ovulation; IUD; or IUS.
5. Patient or legal guardian is able and willing to provide written informed consent and where required assent, and to comply with all requirements of study participation (including all study procedures and continuation of prescribed diet without modification), prior to any screening procedures

9.6.2. Exclusion Criteria

A patient is excluded from this study if he/she has:

1. Clinically significant concurrent disease, serious intercurrent illness, or other extenuating circumstances that, in the opinion of the Investigator, would preclude the patient's participation in the study including:
    a. Had transfusion of ≥ 2 units of RBC within the 60 days before enrollment
    b. Currently has an active infection requiring systemic treatment
    c. Known infection with human immunodeficiency virus (HIV), hepatitis B, or hepatitis C
    d. Currently has uncontrolled hyperammonemia (confirmed ammonia ≥ 100 µmol/L)
    e. Is currently participating in another therapeutic clinical trial
    f. Has received any investigational agent (other than pegzilarginase) within 30 days of enrollment
    g. If female, is lactating or breast feeding

9.6.3. Removal of Patients from Treatment or Assessment

Patients (or their legal guardians) have the right to withdraw from the study at any time for any reason. The Investigator may discontinue a patient from the study for any of the following reasons:

- Occurrence of an AE, development of an intercurrent illness/condition, or change in patient's condition that leads the Investigator to be concerned about the patient's welfare
- Unacceptable toxicity in the judgment of the Investigator
- Patient is uncooperative or noncompliant with study procedures/responsibilities
- Pregnancy in a female patient during the study
- Withdrawal of consent
- Loss to follow-up
- Death

The reason for a patient's withdrawal from the study will be recorded in the electronic case report form (eCRF). Patients who withdraw from study participation for any reason may not re-enter the study at any time.

Patients who are discontinued from the study will be asked to complete follow-up visit procedures.

9.6.4. Replacement of Patients

Patients who withdraw from the study prematurely will not be replaced.

9.7. Treatments

9.7.1. Treatments Administered

9.7.1.1. Initial Dose and Route of Administration

Dosing in an individual patient will commence approximately 4 weeks (but no sooner than 3 weeks) after the last dose of study drug in Study 101A. The pegzilarginase dose level and frequency will initially match the dose the patient was receiving at the end of Study 101A but may be adjusted if clinically indicated in the opinion of the Investigator in consultation with the Sponsor. During the course of the study, doses, regimen (frequency), and route of administration may be adjusted for an individual based on PK/PD data or for safety and/or tolerability reasons as clinically indicated in the opinion of the Investigator in consultation with the Sponsor. Dose adjustments may be made to find a dose and regimen that maintains plasma arginine levels below the target guidance value of 200 µM, and if achievable in the normal range (40 and 115 µM) for as much time as possible during each weekly dosing period. Patients may not receive doses above the maximum tolerated dose (MTD) determined in the oncology trials of 0.33 mg/kg

The first 24 doses in this study will be administered IV. The first 12 doses in this study should be administered using the IV route weekly in the CRU. After the initial 12 IV doses, patients may be dosed IV outside of the CRU (ie, by home health care professionals) if considered safe and appropriate in the opinion of the Investigator in consultation with the Sponsor.

After 24 weeks of IV dosing, patients may be switched to SC dosing weekly if, in the opinion of the Investigator in consultation with the Sponsor, this is considered safe and appropriate for the patient. The initial mg/kg SC dose will be the same as the last IV dose the patient had received. Adjustments to the initial SC dose may be made as clinically indicated based on PK/PD response and any other relevant clinical data. If patients are switched to the SC dosing route, the first 4 SC doses will be given at the investigational site. Subsequent SC injections of the same dose of pegzilarginase may be administered outside of the investigational site by appropriately trained home health care personnel if considered safe and appropriate in the opinion of the Investigator in consultation with the Sponsor.

The Investigator may also switch the patient back to IV dosing at any time if clinically indicated.

Changes in dose level will be made at a clinic visit whenever feasible. On occasion, if a dose change is required and a clinic visit is not feasible, the change may be made on a case-by-case basis after discussion between the Investigator and the Aeglea medical team. A blood sample for PK and PD will be obtained immediately before any dose or route-of-administration change and prior to the 3 subsequent doses at the new dose level for any dose changes that occur in Years 1, 2, or 3 of the study. This will not be required after Week 144. Additional samples for arginine levels may be collected as clinically indicated in the opinion of the Investigator. Samples to evaluate the PK/PD profile will be obtained around the first dose and other timepoints for IV and SC administration according to the PK/PD sampling schedule and Schedules of Assessments (Table 1, Table 2, Table 3, Table 4, Table 5, Table 6,Table 7, and Table 8).

9.7.1.2. Home Health Care

In cases where the patients are dosed outside of the investigational site, appropriately qualified and trained home health care personnel, under the direction of the Investigator, will be responsible for performing the dosing and conducting the safety and efficacy assessments and procedures required by the protocol. These personnel will complete all relevant source documents and provide this information (including safety data) to the Investigator in a timely manner. When appropriate, blood samples may be collected and prepared for shipping to the laboratory by home health care personnel. In instances where this is not appropriate in the opinion of the Investigator (and in consultation with the Sponsor), patients will be required to attend visits at the investigational site. Refer to the Remote Visit Manuals for more detailed information about study visits conducted by home health care personnel.

9.7.2. Identity of Investigational Product

Pegzilarginase is a cobalt-substituted, pegylated, recombinant human Arginase 1. Pegzilarginase drug product will be supplied as a liquid formulation of 2 strengths:

- IV presentation - 10-mL single-use glass vials with a blue cap containing 5 mL of formulated drug product at a concentration of 1 mg/mL for IV administration
- SC presentation – 10-mL single use glass vials with a red cap, containing 6 mL of formulated drug product at a concentration of 5 mg/mL for SC administration
- SC presentation – 10-mL single use glass vials with a green cap, containing 5 mL of formulated drug product at a concentration of 5 mg/mL for SC administration

Pegzilarginase is formulated in 50 mM NaCl, 1 mM K_2HPO_4, 4 mM KH_2PO_4, and 1.5% w/v glycerol.

9.7.3. Storage

Filled vials of pegzilarginase drug product must be stored frozen at or below -60°C.

9.7.4. Directions for Administration

The pegzilarginase dose will be calculated based on the patient's weight.

IV administration: A vial of pegzilarginase 1 mg/mL will be thawed at room temperature. The appropriate amount of pegzilarginase will be withdrawn from the vial and diluted in normal saline. A central venous access port may be considered for patients with poor IV access, at the Investigator's discretion.

SC administration: A vial of pegzilarginase 5 mg/mL will be thawed at room temperature. The appropriate amount of undiluted pegzilarginase will be withdrawn from the vial directly into a syringe that will be used for the SC injection.

Refer to the Pharmacy Manual for complete instructions on pegzilarginase administration.

9.7.5. Method of Assigning Patients to Treatment Groups

This is an open-label study. All patients will receive pegzilarginase.

9.7.6. Selection and Timing of Dose for Each Patient

Details about dosing (including the Sponsor's recommended dosing schedule) are presented in Section 9.7.1.

Pegzilarginase should be administered at approximately the same time of day each time if practical. The Investigator has the discretion to determine the timing of meals in relation to dose for reasons pertaining to safety. However, an approximate 2-hour fast should be observed prior to all sampling for arginine and amino acids and these samples should be collected within 1 hour prior to dose, except where operationally and/or clinically impractical.

Dosing may be paused for an individual patient or a group of participating patients if deemed necessary by the Investigator and the Sponsor. Dosing would resume after review of the safety information and only after agreement by the Investigator and Sponsor. The DSMB may be consulted, if necessary.

9.8. Safety Management

9.8.1. Management of Hypersensitivity Reactions

Patients receiving pegzilarginase may develop a hypersensitivity reaction to either component of the study drug, Co-Arg1 or PEG.

Examples of hypersensitivity reactions include, but are not limited to, flushing, fever and/or chills, pruritus, urticaria, facial and/or tongue edema, chest pain, dyspnea, wheezing, stridor, hypotension or hypertension, bradycardia, or tachycardia.

In the case of a hypersensitivity reaction, an unscheduled sample for ADA assessment will be obtained at the time of the event.

As with any enzyme replacement therapy, medications and equipment for the treatment of hypersensitivity reactions must be available for immediate use in case of unexpected severe hypersensitivity reactions. These supplies include, but are not limited to, oxygen, acetaminophen, antihistamines (eg, diphenhydramine, parenteral and oral), epinephrine, and cardiopulmonary resuscitation devices.

The first 12 IV doses and first 4 SC doses will be administered in a location at the site equipped with immediately accessible resuscitation equipment.

Infusion of study medication should be interrupted in any patient developing signs or symptoms of a hypersensitivity reaction. Hypersensitivity reactions should be treated according to standard of care at the clinical site. Study treatment may be resumed if, in the opinion of the Investigator, the symptoms can be safely managed. Hypersensitivity reactions observed with pegzilarginase in the CAEB1102-101A study were typically managed with antihistamines and a slower infusion rate.

The benefits and risks of corticosteroid treatment should be carefully considered given their potential to cause hyperammonemia in this patient population. Additional information is available in the IB and Pharmacy manual.

The following blood samples will be obtained from any patient who experiences a moderate or severe hypersensitivity reaction within 3 hours of the start of symptoms: serum sample for

tryptase level, serum sample for complement C3, and sample for anti-pegzilarginase antibodies. A second set of samples for tryptase and complement C3 will be obtained 24 hours following the hypersensitivity reaction.

Any patient who experiences a hypersensitivity reaction must safely receive at least 4 subsequent doses in the CRU/hospital/clinic, after which dosing by Home Health Care may be considered if deemed safe by the investigator and after consultation with the sponsor.

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

- Mild reactions are defined as self-limiting, spontaneously-resolving reactions that may be managed with a temporary cessation of dosing or a reduction in the infusion rate.
- 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.

9.8.2. Management of Blood Volume for Pediatric Patients

In trials of pediatric patients, limitations on the amount of blood that can be obtained from an individual are recommended. Per European Union guidance: "Per individual, the trial-related blood loss should not exceed 3% of the total blood volume during a period of 4 weeks and should not exceed 1% at any one time. The total blood volume is estimated at 80 to 90 ml/kg body weight; 3% is 2.4 ml blood per kg of body weight."

Currently, the protocol does not contain any periods where the blood requirements would likely meet criteria for pediatric blood volume limitations. If the limitations are approached, modifications of the blood sampling to limit blood amounts taken may need to be implemented (refer to the Laboratory manual).

9.8.3. Management of Hypoargininemia

Timing for arginine sampling is described in the Schedules of Assessments (Table 1, Table 2, Table 3, Table 4, Table 5, Table 6, Table 7, and Table 8).

Patients will be monitored for any association between low plasma arginine levels and clinically significant symptoms.

9.9. Drug Safety Criteria

Dosing will be interrupted, or subsequent doses postponed as clinically indicated for any patient who:

- Experiences an SAE during dosing that the Investigator believes to be possibly related to pegzilarginase, including any moderate or severe hypersensitivity reaction or hyperammonemia requiring hospitalization.
- Has any clinically significant signs or symptoms which could be attributed to low arginine levels and are temporally related to objective measures of low plasma arginine.

Events requiring interruption of study drug administration will be reviewed by the Sponsor (who may also consult the DSMB). Following this review, treatment may be continued at the discretion of the Investigator following consultation with the Sponsor.

9.10. Prior and Concomitant Medications

Patients are prohibited from receiving other investigational therapies or other enzyme replacement therapy while participating in this study.

Patients experiencing a mild or moderate hypersensitivity reaction or an infusion reaction during or after the first injection may receive prophylaxis prior to subsequent injections.

Patients should receive all necessary supportive care including blood products, transfusions, antibiotics, ammonia scavengers, pain medications, and replacement hormonal therapies (eg, insulin, thyroid hormone, estrogen/progesterone).

No information is available on potential drug-drug interactions with pegzilarginase. Patients must be instructed not to take any medications (over-the-counter, herbals, or prescription) during the study without prior consultation with the Investigator. The Investigator should instruct the patient to notify the study site about any new medications taken after the start of study drug. All medications (other than study drug) and significant non-drug therapies (eg, blood transfusions) taken from the completion of Study 101A through the 30-day safety follow-up visit must be reported on the appropriate eCRF page.

9.11. Treatment Compliance

Patients who are noncompliant with study treatment for reasons other than AEs may be withdrawn from the study.

9.12. Investigational Product Accountability

The Investigator or designee is responsible for maintaining accurate records (including dates and quantities) of IP received, patients to whom IP is dispensed (patient–by–patient dose-specific accounting), and IP lost or accidentally or deliberately destroyed. This responsibility also extends to the home healthcare nurse(s) who will complete drug accountability forms for each dose administered at a patient's home and will provide these records to the site. The Investigator or designee must retain all unused or expired study supplies until the study monitor has confirmed the accountability data. Used study drug vials will be destroyed on-site per the site's standard operating procedures after study drug has been prepared for injection.

Unused study drug must be kept in a secure location for accountability and reconciliation by the study monitor. The Investigator or designee must provide an explanation for any destroyed or missing study drug or study materials.

Unused study drug may be destroyed on site, per the site's standard operating procedures.

All study drug and related materials should be stored, inventoried, reconciled, and destroyed or returned according to applicable state and federal regulations and study procedures.

9.13. Dietary or Other Protocol Restrictions

With the exception of Investigator-directed protein liberalization, patients should remain on the diet prescribed to them at the time of Study 101A completion throughout the entire extension study unless the Investigator or investigative staff directs them to make adjustments. Diet modifications (eg, protein intake liberalization) may be attempted during the course of the study to evaluate how these diet changes may affect certain clinical and biochemical measures. These changes need to be clearly documented in the patients' notes and in the eCRF and 3-day diet record.

9.14. Outcome Measures

The Schedules of Assessments ((Table 1, Table 2, Table 3, Table 4, Table 5, Table 6, Table 7, and Table 8) describe the timing of required evaluations.

9.14.1. Primary Endpoints

Safety and tolerability will be assessed by:

- AEs/SAEs
- Physical examinations
- Vital signs
- ECGs
- Clinical laboratory studies (serum chemistries, hematology, coagulation, urinalysis)
- Low plasma arginine levels associated with clinically significant symptoms
- Clinically significant hyperammonemia
- Immunogenicity safety measures (levels of ADA and anti-PEG antibodies)

9.14.2. Secondary Endpoints

9.14.2.1. Plasma Arginine Levels

Changes in plasma arginine levels after pegzilarginase administration will be assessed.

9.14.2.2. Plasma Guanidino Compounds

Changes in plasma guanidino compound levels after pegzilarginase administration will be assessed.

9.14.2.3. Pharmacokinetics

Pharmacokinetic parameters to be evaluated are time of maximum observed concentration (t_{max}), maximum observed concentration (C_{max}), area under the curve through the time of the last measurable concentration (AUC_{0-t}), area under the curve extrapolated to infinity ($AUC_{0-\infty}$), AUC over the dosing interval (AUC_{0-168}), apparent terminal elimination half-life ($t_{½}$), serum clearance of drug per unit time (CL after IV dosing; CL/F after SC dosing), and volume of distribution (V_{ss} after IV dosing; Vz/F after SC dosing).

9.14.2.4. Neurological/Neuromotor Manifestations

Neurological and neuromotor testing, using functional and spasticity measurements, will include:

- 6MWT
- BBS
- GMFM
- MAS
- Purdue Pegboard Test

9.14.2.5. Other Manifestations of Arginase 1 Deficiency

Other measurements of the impact of the disease will be evaluated by:

- Seizure frequency
- Frequency of hyperammonemic episodes, as measured from laboratory evaluations of ammonia levels and relevant AEs
- Incidence of transaminase elevations, as measured from laboratory evaluations of liver function tests and relevant AEs

9.14.3. Exploratory Endpoints

Exploratory endpoints include:

- Comparison of the PK and PD of pegzilarginase between different routes of administration (IV and SC)
- Changes in exploratory biomarkers after long-term administration of pegzilarginase:
  - Additional amino acids (ornithine, glutamine, leucine, isoleucine, and valine)
  - BUN
- The long-term effects of pegzilarginase on neurocognitive, developmental, and QoL, as selected based on patient age (Table 12), will be measured for the following categories:
  - Intelligence:
    a. Wechsler Adult Intelligence Scale IV
    b. Wechsler Intelligence Scale for Children V
    c. Wechsler Preschool and Primary Scale of Intelligence IV
    d. Bayley Scales of Infant Development III
  - Memory:
    a. Rey Auditory Verbal Learning Test
    b. Atlantis Subtest on the Kaufman Assessment Battery for Children II
  - Attention:
    a. TOVA version 9

- Adaptive Behavior:
  a. Vineland Adaptive Behavior Scales II
- Development:
  b. Growth parameters (length/stature, weight, head circumference)
- QoL:
  a. PROMIS or PedsQL
  Short Form Zarit Burden Interview (ZBI-12)

10. ADVERSE EVENTS

An AE is any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal IP, whether or not considered related to the IP.

An AE may include intercurrent illnesses or injuries that represent an exacerbation (increase in frequency, severity, or specificity) of pre-existing conditions (eg, worsening of asthma). Whenever possible, it is preferable to record a diagnosis as the AE term rather than a series of terms relating to a diagnosis.

The reporting period for nonserious and serious AEs is the period from the signing of consent for the study continuing through the last study follow-up visit. If a nonserious AE remains unresolved at the conclusion of the study, the Investigator and Medical Monitor or designee will make a joint clinical assessment as to whether continued follow up of the AE is warranted, and the results of this assessment must be documented. Resolution is defined as the return to baseline status or stabilization of the condition, with the expectation that it will remain chronic.

The Investigator will assess AEs for severity, for relationship to IP, and whether the event meets one or more of the definitions of an SAE (Section 10.1).

The Investigator will determine the severity of each AE and will record it on the source documents and AE eCRF, using the categories defined in Table 10.

Table 10: Adverse Event Severity Grades

Grade	Description
Mild	No limitation of usual activities
Moderate	Some limitation of usual activities
Severe	Inability to carry out usual activities

The Investigator will determine the relationship of an AE to the IP and will record it on the source documents and AE eCRF, using the categories defined in Table 11.

Table 11: Adverse Event Relationship to Study Drug

Relationship	Description
Not Related	No relationship between the experience and the administration of study drug; related to other etiologies such as concomitant medications or patient's clinical state. Exposure to the IP has not occurred. OR The administration of the IP and the occurrence of the AE are not reasonably related in time. OR The AE is considered likely to be related to an etiology other than the use of the IP; that is, there are no facts [evidence] or arguments to suggest a causal relationship to the IP.

Relationship	Description
Unlikely	The current state of knowledge indicates that a relationship is unlikely. Poor timing, other causes more likely: The administration of the IP and the occurrence of the AE are related in time but the relationship is improbable based on the current state of knowledge AND The AE could be explained equally well by factors or causes other than exposure to the IP. To be used when the exclusion of drug causality of a clinical event seems most plausible
Possibly Related	A reaction that follows a plausible temporal sequence from administration of the study drug and follows a known response pattern to the suspected study drug. The reaction might have been produced by the patient's clinical state or other modes of therapy administered to the patient, but this is not known for sure. Good timing but other causes possible: The administration of the IP and the occurrence of the AE are reasonably related in time. AND The AE could not be explained equally well by factors or causes other than exposure to the IP. To be used when drug causality is one of the other possible causes
Probably Related	A reaction that follows a plausible temporal sequence from administration of the study drug and follows a known response pattern to the suspected study drug. The reaction cannot be reasonably explained by the known characteristics of the patient's clinical state or other modes of therapy administered to the patient. Good timing, other causes unlikely, withdrawal challenge. The administration of IP and the occurrence of the AE are reasonably related in time. AND The AE is more likely explained by exposure to the IP than by other factors or causes. AND The AE follows a clinically reasonable response on withdrawal (dechallenge).
Definitely Related	A reaction that follows a plausible temporal sequence from administration of the study drug and follows a known response pattern to the suspected study drug and can be confirmed with a positive re- challenge test or supporting laboratory data.

AE = adverse event; IP = investigational product.

In order 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.

10.1. Serious Adverse Events

An SAE is defined as any AE that:

- Results in death.
- Is life threatening, that is, places the patient at immediate risk of death from the event as it occurs. This definition does not include a reaction that, had it occurred in a more severe form, might have caused death.

- Requires in-patient hospitalization or prolongation of an existing in-patient hospitalization.
- 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 patient's ability to carry out usual life functions. This definition is not intended to include experiences of relatively minor or temporary medical significance.
- Is a congenital anomaly or birth defect, ie, an AE that occurs in the child or fetus of a patient exposed to IP prior to conception or during pregnancy.
- Is an important medical event or serious medical condition that does not meet any of the above criteria but may jeopardize the patient or require medical or surgical intervention to prevent 1 of the outcomes listed above. Examples of such events are intensive treatment in the emergency room, allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

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

The reporting period for SAEs is the period from the signing of the consent form for the study continuing through the last study follow-up visit. SAEs reported to the Investigator outside of this reporting period will be reported to Aeglea and the Medical Monitor if, in good medical judgment, the event has any bearing on the study data. SAEs must be followed by the Investigator until resolution, even if this extends beyond the study reporting period. Resolution of an SAE is defined as the return to baseline status or stabilization of the condition, with the expectation that it will remain chronic.

Any SAE, whether or not considered related to study drug, will be reported immediately (within 24 hours) to Aeglea or its Safety designee by fax or scan and email using the study-specific SAE Report Form. In addition to the outcome of the SAE, any medication or other therapeutic measures used to treat the event will be recorded on the appropriate eCRF page(s). Investigators should not wait to collect additional information that fully documents the event before notifying Aeglea or its Safety designee of an SAE. Aeglea 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 Aeglea, the Medical Monitor, and/or their Safety designee as soon as it becomes available.

Reporting of SAEs to the IRB/EC will be done in compliance with the standard operating procedures and policies of the IRB/EC and with applicable regulatory requirements. Adequate documentation must be obtained by Aeglea showing that the IRB/EC was properly and promptly notified as required.

A completed SAE Report form signed by the Investigator must be transmitted to the Sponsor's Safety representative at [redacted] via fax at [redacted] or scanned and emailed to [redacted]. The SAE may also be reported by telephone to the [redacted]. However, if the SAE is reported by telephone, the completed SAE form must be submitted as noted above within 24 hours of telephone notification. [redacted] will forward the SAE Report form to Aeglea and the Medical Monitor.

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 as follows:

[redacted]

[redacted]

Aeglea BioTherapeutics, Inc.
805 Las Cimas Pkwy
Suite 100, Building III
Austin, TX 78746

Office: [redacted]
Cell: [redacted]
Email: [redacted]

11. DEFINITION OF STUDY PROCEDURES

Study procedures should be performed according to the Schedules of Assessments (Table 1, Table 2, Table 3, Table 4, Table 5, Table 6, Table 7, and Table 8).

11.1. Informed Consent

Written informed consent and, where appropriate, assent, must be obtained from the patient or the patient's legal guardian before any study-related procedures are performed and whenever the study procedures change or new safety information becomes available that may affect the patient's willingness to participate.

11.2. Demographics and Medical History

Demographics and medical history information recorded for the 101A study will be updated as appropriate upon patient entry into this study.

11.3. Physical Examination

Complete, age-appropriate physical examinations will be performed by the Investigator or qualified designee at the timepoints specified in the Schedules of Assessments (Table 1, Table 2, Table 3, Table 4, Table 5, Table 6, Table 7, and Table 8). The physical examination will include evaluation of the head, eyes (sclera, conjunctiva, pupillary reflexes, extraocular muscles, non-dilated fundoscopic assessment), ears, nose, throat, cardiovascular, dermatological, musculoskeletal, respiratory, gastrointestinal, and neurological systems. Neurological examination must include level of consciousness, mental status, speech, vision, cranial nerves III, IV, and VI, motor, sensory and limb ataxia assessments. For male patients, careful attention (when practical) should be given to signs or symptoms suggestive of a potential drug-induced change in the male reproductive system. Further assessment, including urological referral, would be encouraged.

Weight will be assessed during the baseline period and approximately every 4 weeks during dosing until Week 96. After Week 96 weight will be assessed every 12 weeks during dosing. Baseline weight can be used to calculate total dose, unless subsequent measures of weight show a > 10% change. In the event the weight changes > 10%, then the subsequent weight will be used to calculate the total dose.

For adults, height will be collected at baseline only. For pediatric patients, height will be collected for the clinical growth assessments (Section 11.5.5.1).

No rectal or pelvic examinations are required.

11.4. Neurological/Neuromotor Function Testing

In addition to the neurologic examination completed with the physical examination, several assessments of neurological and neuromotor function will be tested: the 6MWT; the BBS; the GMFM, the MAS, and the Purdue Pegboard Test. The timing of these assessments is specified in the Schedules of Assessments (Table 1, Table 2, Table 3, Table 4, Table 5, Table 6, Table 7, and Table 8). All neurological/neuromotor evaluations should be administered by appropriately qualified professionals and performed consistently between visits and clinical sites. Assessments

may be conducted over more than 1 day. Where allowed, these assessments may be videotaped at baseline and at subsequent timepoints thereafter.

11.4.1. Six-Minute Walk Test

The 6MWT has been widely used in clinical studies to globally assess the cardiovascular, pulmonary, and neuromuscular systems in numerous disease conditions. It measures the distance a patient can walk on a flat surface in 6 minutes. The American Thoracic Society (ATS) published a standardized methodology for performing the test, which is well tolerated and simple to administer. The standardized methodology as defined by the ATS (ATS 2002) will be followed. This assessment should be performed before any other assessments conducted on a given day. Patients will be instructed to wear shoes that securely cover their entire foot (eg, sneakers, etc.) rather than sandals or slippers, etc.

Modifications of the methodology may be allowed to account for the disease state of the patient as well as logistic considerations of the testing location, with approval from the Sponsor.

Non-ambulatory patients will not have the 6MWT administered. Results of the 6MWT will be recorded as distance completed in meters and percent change from baseline.

11.4.2. Berg Balance Scale

The BBS measures balance in patients with impairment of balance function by assessing performance in certain functional tasks. There are 14 tasks evaluating certain aspects of balance such as sitting to standing, transfers, turning, and standing on 1 foot. Additionally, most tasks require the subject to maintain the position for a specific time.

11.4.3. Gross Motor Function Measure

The GMFM is a clinical measure designed to evaluate changes in gross motor function, looking at a range of activities such as lying, rolling, walking, running, and jumping. A 4-point scoring system is used for each item of the GMFM covering 5 dimensions: lying and rolling; sitting; crawling and kneeling; standing; walking, running, and jumping.

11.4.4. Modified Ashworth Scale

The MAS was developed to assess the spasticity of patients with central nervous system lesions and is used to measure the resistance to passive movement about a joint due to spasticity. The scale utilizes a scoring scale of 0 (no spasticity) to 4 (total rigidity) with 6 scoring choices as shown below:

Score	Modified Ashworth Scale
0	No increase in tone
1	Slight increase in tone manifested by a catch and release or by minimal resistance at the end of the ROM when moved in flexion or extension
1+	Slight increase in tone manifested by a catch, followed by a minimal resistance throughout the remainder (less than half) of the ROM
2	More marked increase in tone through most of the ROM, but affected parts easily moved
3	Considerable increase in tone; passive movement difficult
4	Limb rigid in flexion or extension
ROM = Range of Motion	

The test is well tolerated and easy to perform. Care should be taken as the scale cannot differentiate between spasticity and soft tissue stiffness.

11.4.5. Purdue Pegboard Test

The Purdue Pegboard Test is a test of manipulative dexterity and bimanual coordination measuring performance speed in a fine motor task utilizing both sides of the body (Tiffin 1948). The test involves 2 different abilities: gross movements of arms, hands, and fingers, and fine motor extremity, also called "fingerprint" dexterity. Poor pegboard performance is a sign of deficits in complex, visually guided, or coordinated movements that are likely mediated by circuits involving the basal ganglia.

11.4.6. Seizure Frequency

Patients will record all seizures during the course of the study. The date and (approximate) time of the seizure should be noted. Collection of seizure frequency information will begin at baseline and continue throughout the study.

11.5. Neurocognitive, Developmental, and Quality of Life (QoL) Assessments

Neurocognitive, developmental, and QoL tests to evaluate the intelligence, memory, attention, adaptive behavior, growth/development, and QoL of patients are presented in Table 12 and will be assessed at the timepoints indicated in the Schedule of Assessments (Table 1, Table 2, Table 3, Table 4, Table 5, Table 6, Table 7, and Table 8). With the exception of the VABS-II, the PROMIS or PedsQL, and the ZBI-12, which are assessments administered to the patient (as appropriate) or to the parent or caregiver, all other assessments will be administered only to the patient (as is age-appropriate). As is feasible, these assessments may be videotaped at baseline and at subsequent timepoints thereafter.

All evaluations should be administered by appropriately qualified professionals and consistently between visits and clinical sites. Assessments may be conducted over more than 1 day. The selection of the appropriate test instrument administered to a patient is normally dependent on the calendar age of the patient. For assessments that may overlap by age in any one domain, the

assessor will evaluate the patient's functional capabilities to determine which test is most appropriate for the patient. The assessor will always attempt to administer the most advanced age-appropriate test to a given patient. If the assessor determines during administration of the assessment that the patient's functional capabilities are not appropriate for the more advanced test, they will end the assessment with the advanced test and administer the less advanced test instead.

Table 12: CAEB1102-102A Neurocognitive, Developmental, and Quality of Life Assessments

Domain Assessed	Test Name	Description	Age Range	Approximate Time Required
Intelligence	Wechsler Adult Intelligence IV (WAIS-IV)	Verbal and Performance intelligent quotients (IQs) are yielded and Full Scale IQ can be measured. Processing speed and memory are also measured. Shortened version of Full Scale IQ without processing speed and working memory called the General Ability Index (GAI) may be used.	16 years and older	30 to 40 minutes
	Wechsler Intelligence Scale for Children V (WISC-V)	Verbal and Performance IQs are yielded and Full Scale IQ can be measured. Processing speed and memory are also measured. Shortened version of Full Scale IQ without processing speed and working memory (the GAI) may be used.	6 to 16 years	45 to 65 minutes
	Wechsler Preschool and Primary Scale of Intelligence IV (WPPI-IV)	Verbal and Performance IQs are yielded as well as Full Scale IQ. Processing speed and memory is also measured. Shortened version of Full Scale IQ without processing speed and working memory (the GAI) may be used.	3 to 6 years	30 to 40 minutes
	Bayley Scales of Infant Development III (BSID-III)	Developmental test of cognitive ability for ages 2 to 3.5 years. Cognitive Scale and Mental Development Index will be included as an endpoint. Mental Development Index is the score. Motor component will be included. Language scales should also be given but will be exploratory only. This will be the only direct measurement in children younger than 4 years of age.	2 to 3.5 yea rs	40 minutes
Memory	Rey Auditory Verbal Learning Test (RAVLT)	A list of 15 unrelated words is read aloud. The number of words recalled after each of 3 trials and a 30-minute delay is recorded.	6 years and older	40 to 45 minutes

Domain Assessed	Test Name	Description	Age Range	Approximate Time Required
	Atlantis Subtest on the Kaufman Assessment Battery for Children II (KABC-II)	A set of pictures are paired with nonsense words; child must recognize picture paired with word on both learning and delayed response trials.	3 to 8 years	20 minutes
Attention	Test of Variables of Attention (TOVA) version 9	Test requires the child/adult to respond with a button or key on a keyboard to a designated stimulus that is flashed on the screen and not to respond to other stimuli. The speed, accuracy, and consistency are measured.	6 years to adult	22 minutes
Development	Gender- and age-specific growth charts from the Centers for Disease Control (CDC)	Stature-for-age and weight-for-age charts will be collected, and weight-for-stature will additionally be collected for patients 2 to 5 years old. Age-appropriate head circumference measures will be collected.	≥ 2 to < 18 years	Varies
Patient or Parent Reported Outcomes				
Adaptive Behavior	Vineland Adaptive Behavior Scales II (VABS-II)	Parent observational measure by interview format will be used. Includes composite score, communication, daily living skills, socialization, and motor skills.	All ages	30-60 minutes
Quality of Life	Patient-Reported Outcomes Measurement Information System (PROMIS) Parent Proxy v2.0 PROMIS Pediatric Profile v2.0 PROMIS 29 Pediatric Quality of Life Inventory (PedsQL) Measurement Model for the Pediatric Quality of Life Inventory	Rating of QoL.	2 to 18 years	5-10 minutes
	Short Form Zarit Burden Interview (ZBI-12)	Parent/caregiver self-rating of QoL.	All ages	30 minutes

11.5.1. Assessments of Intelligence

11.5.1.1. Wechsler Adult Intelligence Scale IV

The WAIS test has 10 core subtests and 5 supplemental subtests (Wechsler 2008). The test measures abilities in 4 main categories. These include reasoning, retention of information,

processing and organization of information, and verbal comprehension. Factors such as creativity, individuality, or judgment are not incorporated into the test. Each category is scored individually, and the composite score is used to obtain the intelligence quotient (IQ), simply referred to as IQ.

The General Ability Index (GAI) for this test consists of the Similarities, Vocabulary, and Information subtests from the Verbal Comprehension Index and the Block Design, Matrix Reasoning, and Visual Puzzles subtests from the Perceptual Reasoning Index. The GAI is clinically useful because it can be used as a measure of cognitive abilities that are less vulnerable to impairments of processing and working memory.

11.5.1.2. Wechsler Intelligence Scale for Children V

The WISC-V generates a Full Scale IQ (formerly known as an intelligence quotient or IQ score) that represents a child's general intellectual ability (Wechsler 2014). It also provides 5 primary index scores: Verbal Comprehension Index, Visual Spatial Index, Fluid Reasoning Index, Working Memory Index, and Processing Speed Index. These indices represent a person's abilities in discrete cognitive domains. Five ancillary composite scores can be derived from various combinations of primary or primary and secondary subtests.

The GAI for this test provides the practitioner a summary score that is less sensitive to the influence of working memory and processing speed. The GAI is calculated from the General Ability Sum of Scaled Scores, which is the sum of scaled scores for 3 Verbal Comprehension subtests (ie, Vocabulary, Comprehension, and Similarities) and 3 Perceptual Reasoning subtests (ie, Block Design, Matrix Reasoning, and Picture Concepts).

11.5.1.3. Wechsler Preschool and Primary Scale of Intelligence IV

The WPPSI is an intelligence test designed for children ages 2 years 6 months to 7 years 7 months. WPPSI–IV is a revision of the WPPSI-R (Wechsler 1989) and the WPPSI-III (Wechsler 2002). It provides subtest and composite scores that represent intellectual functioning in verbal and performance cognitive domains, as well as a composite score that represents a child's general intellectual ability (ie, full scale IQ). It consists of 13 subtests (block design, information, matrix reasoning, bug search, picture memory, similarities, picture concepts, cancellation, zoo locations, object assembly, vocabulary, animal coding, and comprehension). They are designated as 1 of 3 types: core, supplemental, or optional. The core subtests are required for the computation of the verbal, performance, and Full Scale IQ.

The WPPSI–IV provides verbal and performance IQ scores as well as a Full Scale IQ score. In addition, the processing speed quotient can be derived for children aged 4 to 7 years 7 months, and a general language composite can be determined for children in both age bands (2 years 6 months to 3 years 11 months and 4 to 7 years 7 months). Children in the 2 years 6 months to 3 years 11 months age band are administered 6 subtests: receptive vocabulary, block design, picture memory, information, object assembly, and zoo locations.

11.5.1.4. Bayley Scales of Infant and Toddler Development III

The BSID-III has been validated for children ages younger than 3.5 years (Connolly 2012). The test is administered to the subject and was designed to identify young children with development delays. The BSID-III assesses developmental function across 5 domains: cognition; language

(expressive and receptive); motor (fine and gross motor functioning); social-emotional; and adaptive behavior.

11.5.2. Assessments of Memory

11.5.2.1. Rey Auditory Verbal Learning Test

The RAVLT (Rey 1958; Schmidt 2004) evaluates a wide diversity of functions: short-term auditory-verbal memory, rate of learning, learning strategies, retroactive, and proactive interference, presence of confabulation of confusion in memory processes, retention of information, and differences between learning and retrieval.

Participants are given a list of 15 unrelated words repeated over 5 different trials and are asked to repeat. Another list of 15 unrelated words are given and the subject must again repeat the original list of 15 words and then again after 30 minutes. Approximately 10 to 15 minutes is required for the procedure (not including the 30-minute interval).

11.5.2.2. Atlantis Subtest on the Kaufman Assessments Battery for Children II

The KABC-II has been validated for children aged 3 to 18 years (Gallagher 2011). The test is administered to the subject and was designed to identify strengths and weaknesses in cognitive ability and mental processing. The KABC-II is comprised of 18 subtests (although not all subtests are required to be administered), and the test is typically used in conjunction with other cognitive or neuropsychological assessments. One of these subtests evaluates long-term retrieval, which is the ability to store and efficiently retrieve newly learned or previously learned information. A component of this scale is the Atlantis Subtest, which consists of identifying pictures of fish, plants, and shells when given nonsense names by the examiner to evaluate long-term retrieval.

11.5.3. Assessments of Attention

11.5.3.1. Test of Variables of Attention Version 9

The TOVA (Greenberg 1991; Greenberg 2017) uses geometric stimuli (to minimize the effects of cultural differences and learning problems) and contains two test conditions: target infrequent and target frequent. In the first half of the test (the target infrequent half), a target is presented (randomly) only once every 3.5 nontarget presentations. In this half, which is similar to most of the other continuous performance tests, the task is boring and fatiguing, and the subject must pay close attention to respond to the infrequent target correctly. When a subject does not respond to the target, it is called an error of omission and is a measure of inattention. In the second half of the test (target frequent half), 3.5 targets are presented for every 1 nontarget. In this half of the test, the subject expects to respond most of the time but occasionally must inhibit the tendency to respond. When a subject responds to the nontarget, it is called an error of commission and is a measure of impulsivity. Thus, the ability to pay attention to a boring, repetitive task is best measured in the first half of the TOVA, while the ability to inhibit oneself is best measured in the second half. TOVA measures include variability of response time (consistency), response time, commission (impulsivity), errors of omission (inattention), post-commission response times, multiple and anticipatory responses, and an attention-deficit/hyperactivity disorder (ADHD) score, which is a comparison to an age/gender specific ADHD group.

11.5.4. Assessments of Adaptive Behavior

11.5.4.1. Vineland Adaptive Behavior Scales II

The Vineland II is a scale designed to measure adaptive behavior of individuals from birth to age 90 (Sparrow 2005). The Vineland-II contains 11 general subdomains, grouped into 4 domains: communication, daily living skills, socialization, and motor skills. The domains are made up of subdomains in which the scores are added to form the domain composite scores. The 4domain composite scores then combine to form the adaptive behavior composite for those individuals aged birth to 6 years 11 months. Three domain composite scores (communication, daily living skills and socialization) combine to form the adaptive behavior composite for those aged 7 through 90.

11.5.5. Assessments of Development

11.5.5.1. Clinical Growth Assessments

Clinical growth charts provide a tool for evaluating the growth of children in clinical research settings. The gender- and age-specific Centers for Disease Control and Prevention (CDC) growth charts (CDC 2002) will be used for the study. Only pediatric patients ages ≥ 2 to < 18 years old participating in the study will have these assessments done. Stature-for-age and weight-for-age charts will be collected for all pediatric patients, and weight-for-stature will additionally be collected for patients 2 to 5 years old. Age-appropriate head circumference measures will be collected as well.

11.5.6. Quality of Life Assessments

11.5.6.1. Measurement Model for the Pediatric Quality of Life Inventory

The PedsQL Measurement Model for the Pediatric Quality of Life Inventory is a modular approach to measuring health-related quality of life (HRQoL) in healthy children and adolescents and those with acute and chronic health conditions. The PedsQL Measurement Model integrates seamlessly both generic core scales and disease-specific modules into one measurement system.

The 23-item PedsQL Generic Core Scales were designed to measure the core dimensions of health as delineated by the World Health Organization, as well as role (school) functioning.

The 4 Multidimensional Scales are Physical Functioning (8 items) Emotional Functioning (5 items) Social Functioning (5 items) School Functioning (5 items).

The 3 Summary Scores are: Total Scale Score (23 items), Physical Health Summary Score (8 items), Psychosocial Health Summary Score (15 items).

The tool is practical and can be completed in 5 minutes. It is developmentally appropriate [Ages 2-18]: Child self-report Ages 5-7, 8-12, 13-18; Parent Proxy Report: Ages 2-4, 5-7, 8-12, 13-18. The investigator/ testing psychologist will determine whether a child is able to complete the self-report at study baseline. For children where they are not able, or it is not appropriate for them to complete the self-report eg children 2-4 years old, the Parent Proxy- report will be used.

11.5.6.2. Patient-Reported Outcomes Measurement Information System

The PROMIS is a set of person-centered measures that evaluates and monitors physical, mental, and social health in adults and children. It can be used with the general population and with individuals living with chronic conditions. The Pediatric/Parent Proxy Profile is a collection of 4 item short forms assessing anxiety, depressive symptoms, fatigue, pain interference, physical function-mobility, and peer relationships as well as a single pain intensity item. PROMIS-29 is a collection of 4-item short forms assessing anxiety, depression, fatigue, pain interference, physical function, sleep disturbance, and ability to participate in social roles and activities as well as a single pain intensity item.

11.5.7. Short Form Burden Interview

The ZBI (Zarit 1985) is a widely used tool to describe caregiver burden and has been well validated in various caregiving samples. The ZBI-12 (Bédard 2001) is a 12-item short version of the ZBI. The scores from the ZBI-12 have previously been assessed to have good correlation with the original version in caregivers of advanced cancer patients. All versions feature statements that are ranked by caregiver as occurring never, rarely, sometimes, quite frequently, or nearly always.

11.6. Clinical Laboratory Tests

Laboratory samples will be obtained according to the Schedules of Assessments (Table 1, Table 2, Table 3, Table 4, Table 5, Table 6, Table 7, and Table 8). The following tests will be obtained for this study:

- Hematology: RBCs, white blood cells (WBCs), hemoglobin, hematocrit, WBC differential count (neutrophils, lymphocytes, eosinophils and basophils), platelet count, reticulocyte count, mean corpuscular hemoglobin concentration, mean corpuscular hemoglobin, mean corpuscular volume, mean platelet volume, and RBC distribution width.
- Serum chemistry: ammonia, sodium, potassium, chloride, bicarbonate, calcium, phosphate, BUN, creatinine, glucose, lactate dehydrogenase, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, total bilirubin, total protein, and albumin.
- Urinalysis: appearance, glucose, ketones, blood, protein, nitrate, bilirubin, specific gravity, pH, urobilinogen, and leukocytes. If positive for blood or protein trace, then include microscopy.
- The prothrombin (PT)/international normalized ratio (INR) and activated PT time (aPTT) tests will be performed to assess the clotting ability of the patient's blood. The INR will be calculated by the laboratory using the PT ratio and the appropriate International Sensitivity Index value of the thromboplastin reagents used.
- Serum or urine pregnancy tests will be performed for all female patients of childbearing potential at the timepoints described in the Schedules of Assessments (Table 1, Table 2, Table 3, Table 4, Table 5, Table 6, Table 7, and Table 8). Patients who have a confirmed positive pregnancy test at any time during the study will be

discontinued from further study drug administration and will be followed for pregnancy outcome.

- Serum tryptase and serum complement C3 will be obtained at baseline and in the event of a hypersensitivity reaction. Samples should be obtained no later than 3 hours after the start of the reaction and at 24 hours after the start of the reaction.

Clinically significant abnormal laboratory test results should be followed until a return to normal or baseline values. Details for the collection, processing, storage, and shipment of samples can be found in the study laboratory manual.

11.7. Pharmacodynamic (PD) Samples

Blood samples for PD analyses, including plasma arginine and ornithine, will be drawn at timepoints designated in the Schedules of Assessments (Table 1, Table 2, Table 3, Table 4, Table 5, Table 6, Table 7, and Table 8). Note that a local laboratory assessment of plasma arginine for safety purposes is permitted; however, the result must be interpreted with caution as the value may be spuriously low during pegzilarginase treatment. This local laboratory assessment, should such be used, should not replace the use of the designated central laboratory and should only be used for informational purposes. This study uses specific sampling tubes with an arginase inhibitor that must be analyzed at the designated reference laboratory to facilitate accurate assessment of plasma arginine. For consistency of data, local laboratory samples should also be sent to the central laboratory. PD analyses may also be conducted via dry blood spot (DBS) by fingerstick or via alternate methods as outlined in the lab manual once they are available and will be sent to a designated central laboratory.

11.8. Other Amino Acids

Blood samples for amino acid analysis including glutamine, leucine, isoleucine, and valine will be collected at the times designated in the Schedules of Assessments (Table 1, Table 2, Table 3, Table 4, Table 5, Table 6, Table 7, and Table 8). An approximate 2-hour fast should be observed prior to all sampling for amino acids and within 1 hour prior to dose, except where operationally and/or clinically impractical. The time of sampling and date and time of each pegzilarginase dose will be recorded. Samples will be shipped to the central laboratory as detailed in the laboratory manual.

11.9. Plasma Guanidino Compounds

Blood samples will be collected to evaluate the exposure of patients to GCs in an exploratory manner. Sampling times will be the same as for arginine as listed in the Schedules of Assessments (Table 1, Table 2, Table 3, Table 4, Table 5, Table 6, Table 7, and Table 8). Potential GCs to be collected may include guanidinoacetic acid, α-N-acetylarginine, α-keto-δ-guanidinovaleric acid, argininic acid, creatine, homoarginine, or others.

11.10. Pharmacokinetic Samples

Blood samples for PK analysis will be drawn as described in the Schedules of Assessments (Table 1, Table 2, Table 3, Table 4, Table 5, Table 6, Table 7, and Table 8).The actual time and

date of each PK sampling and start and stop times of each pegzilarginase administration will be recorded. Samples will be shipped to and stored at the bioanalytical laboratory at ≤ -70°C during the course of the study.

11.10.1. Anti-drug Antibody Testing

Blood samples for ADA testing will be drawn as described in the Schedules of Assessments (Table 1, Table 2, Table 3, Table 4, Table 5, Table 6, Table 7, and Table 8). Samples will be analyzed both for antibodies against pegzilarginase and against PEG. Samples will be shipped to and stored at the bioanalytical laboratory at ≤-70°C during the course of the study. An unscheduled sample for ADA assessment will be obtained in the event of a moderate or severe hypersensitivity reaction. Patients who test positive for ADA or neutralizing antibodies will be followed and re-tested approximately every 2 to 3 months until the antibody levels return to pre-exposure levels.

11.11. Three-Day Diet Record

Throughout the study, patients will complete a 3-day diet record using a diary to collect the information prior to dosing every 4 weeks beginning prior to the first dose at Week 1. Between Week 96 – Week 144, a 3-day diet record will be collected every 12 weeks per the Schedules of Assessments (Table 1, Table 2, Table 3, Table 4, Table 5, Table 6, Table 7, and Table 8). After Week 144,the 3-day diet record will not be collected except at the EOT/SFU visit. The Investigator or dietician should review the patients' entries to verify diet, if possible. The total calories and total protein, including natural protein, medical food protein, and essential amino acids, should be recorded on the source documentation. The prescribed diet should be recorded in the source document and eCRF for each 3-day diet record (ie, grams of protein per kg of body weight and calories per kg of body weight).

11.12. Vital Signs

Vital signs (including blood pressure, pulse, respirations, and temperature) will be assessed at the timepoints specified in the Schedules of Assessments (Table 1, Table 2, Table 3, Table 4, Table 5, Table 6, Table 7, and Table 8).

11.13. Electrocardiogram

A 12-lead ECG will be obtained after 5 minutes of rest in the supine position using equipment at the site at the visits noted on the Schedules of Assessments (Table 1, Table 2, Table 3, Table 4, Table 5, Table 6, Table 7, and Table 8). An unscheduled ECG should be obtained as clinically indicated for cardiovascular symptoms. The Investigator or designee will evaluate the ECG for abnormalities.

11.14. Follow-Up

All patients, including patients who withdraw from the study prematurely, will return to the study site for a follow-up visit approximately 2 weeks following the last dose of study drug. Follow-up visit procedures are noted in the Schedules of Assessments (Table 8).

If a patient fails to return for scheduled visits, a documented effort must be made to determine the reason. If the patient cannot be reached by telephone after 2 attempts, a certified letter should be sent to the patient (or the patient's legally authorized representative, if appropriate) requesting contact with the Investigator. This information should be recorded in the study records.

12. DATA QUALITY ASSURANCE

Aeglea BioTherapeutics, Inc. personnel (or designee) will visit the study sites prior to initiation of the study to review with the site personnel information about the IP, protocol, and other regulatory document requirements, any applicable randomization procedures, source document requirements, eCRFs, monitoring requirements, and procedures for reporting AEs, including SAEs.

At visits during and after the study, the Sponsor and/or designee will monitor the site for compliance with regulatory documentation, with a focus on accurate and complete recording of data on eCRFs from source documents, adherence to protocol, randomization (if applicable), SAE reporting, and drug accountability records.

A Sponsor-designated clinical data management group will design and program a study database and corresponding eCRFs and provide training for sites and CRAs on data entry and cleaning procedures. Data quality control and analysis will be performed by Aeglea (or designee), including study monitors and the clinical data management group, based on a predefined data management plan as well as a statistical analysis plan (SAP).

13. STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

13.1. Statistical Analysis Plan

The SAP will provide a detailed description of the planned statistical analyses. The SAP will be finalized prior to database lock. Any changes to the methods set forth in the SAP will be described in the final clinical study report.

This study is an extension of Study 101A, and the results from Study 101A may be included in analyses of results from this study.

13.2. Analysis Sets

- Full Analysis Set (FAS): all patients who receive any study medication. This analysis set is consistent with the intent-to-treat (ITT) principle and will be used for safety analyses. This analysis set will include all Study 101A data for the patients who have received at least 1 dose of pegzilarginase in the current study.
- Pharmacokinetic (PK) Set: all patients in the FAS with quantifiable serum concentrations of pegzilarginase and who provide sufficient serum concentrations to support PK parameter estimation (in either Study 101A or the current study).

13.3. Missing Data Handling

Imputation of missing data is not planned. In the case of missing data, supportive analyses may be performed using selected imputation schemes; such analyses will be described in the clinical study report.

13.4. Data Analysis Conventions

Most analyses will be descriptive, presenting the data using summary statistics and/or graphs. Statistical tests may be performed as part of the descriptive analyses, as a measure of observed effects of pegzilarginase relative to observed variability. Such tests will be performed at 2-sided $\alpha = 0.05$ unless otherwise stated and will not be adjusted for multiplicity. The term "statistically significant" may be used to describe results where the p-value is no greater than 0.05; this phrase will be considered to mean "nominally statistically significant" and will not imply a prespecified hypothesis test.

13.5. Disposition of Patients

The number and percentage of patients entering and completing the clinical study will be presented, along with primary reason for discontinuation of any patients who do not complete the study. A flowchart of patient disposition, similar to a CONSORT diagram for randomized controlled studies, may be created. In addition, the number and percentage of patients discontinuing from treatment will be tabulated by reason for discontinuation.

13.6. Demographic and Baseline Characteristics

Demographics and baseline characteristics for patients in this study will be summarized from the data from Study 101A. Note that not all patients from Study 101A may participate in the current study, so the demographic and baseline characteristics tables from Study 101A and the current study may differ. A comparison of baseline characteristics from Study 101A that were reassessed during screening for the current study may be presented.

13.7. Primary Endpoint Analysis

All AEs will be listed and summarized. The number and percentage of patients with AEs and treatment-related AEs; SAEs and treatment-related SAEs; discontinuations due to an AE; and fatal AEs will be summarized by MedDRA system organ class and preferred term. Similarly, descriptive summaries by dose (ie, dose immediately preceding the AE) for the various categories of AEs may be created. Summaries of AEs by maximum severity will also be produced.

Vital signs will be listed and summarized across time. Abnormal changes in ECGs and physical examinations will be listed.

Laboratory data will be listed and summarized across time; values outside the normal range will be flagged. Primary laboratory studies will include hematology, chemistry, urinalysis, coagulation, plasma arginine levels, and ammonia levels. Immunogenicity safety measures will include levels of ADA and anti-PEG antibodies. For any patients who develop low plasma arginine levels associated with clinically significant symptoms, clinically significant hyperammonemia, and or ADAs to either pegzilarginase or PEG, listings will be provided for temporally related AEs and laboratory data (arginine, ammonia, antibody titers, and PK data).

13.8. Secondary Endpoint Analyses

13.8.1. Plasma Arginine Levels

On-treatment arginine levels will be summarized. Each patient will serve as his/her own control. Arginine levels will also be listed across time. The relationship between arginine and other endpoints may be described graphically, such as with scatterplots. Additional mathematical modeling of arginine data may be performed.

13.8.2. Plasma Guanidino Compounds

Levels of guanidino compounds will be listed and summarized across time. Values outside the normal range will be flagged.

13.8.3. Pharmacokinetic Analyses

Due to sparse PK sampling, data analyses will be limited to exploratory graphical and /or tabular summaries. Details will be provided in the SAP or in a separate related document.

13.8.4. Neurologic and Neuromotor Manifestations

Results from the neurologic and neuromotor testing results will be summarized; details will be provided in the SAP or in a separate related document.

13.8.5. Other Manifestations

Seizure frequency, frequency of hyperammonemic episodes, and incidence of disease-related transaminase elevations will be summarized; details will be provided in the SAP or in a separate related document.

13.9. Exploratory Analyses

13.9.1. Comparison of Pharmacokinetics and Pharmacodynamics of Pegzilarginase by Dosing Schedule and Route

Details of the planned comparison of the PK and PD of pegzilarginase between different routes of administration (IV and SC) will be provided in the SAP or in a separate related document.

13.9.2. Changes in Exploratory Biomarkers

Levels of additional amino acids (ornithine, glutamine, leucine, isoleucine and valine) and BUN will be listed and summarized across time. Values outside the normal range will be flagged.

13.9.3. Neurocognitive, Developmental, and Quality of Life Assessments

Details of the analysis of data related to neurocognitive, developmental, and QoL assessments, as selected based on patient age, will be described in the SAP or in a separate related document.

13.10. Determination of Sample Size

The sample size of approximately10 pediatric and adult patients was determined using clinical, rather than statistical, considerations. This sample size may lack power to distinguish a statistically significant result.

13.11. Multiplicity Considerations

Not applicable.

13.12. Changes in the Conduct of the Study or Planned Analyses

Only Aeglea BioTherapeutics, Inc., may modify the protocol. Any change in study conduct considered necessary by the Investigator will be made only after consultation with Aeglea, who will then issue a formal protocol amendment to implement the change. The only exception is when an Investigator considers that a patient's safety is compromised without immediate action. In these circumstances, immediate approval of the Chairman of the IRB/EC must be sought, and the Investigator should inform Aeglea and the full IRB/EC within 2 working days after the emergency occurs.

Aeglea may conduct interim analyses as needed to support regulatory filings.

With the exception of minor administrative or typographical changes, the IRB/EC must review and approve all protocol amendments. Protocol amendments that have an impact on patient risk or the study objectives, or require revision of the ICF, must receive approval from the IRB/EC prior to their implementation.

When a protocol amendment substantially alters the study design or the potential risks or burden to patients, the ICF will be amended and approved by Aeglea and the IRB/EC, and all active patients must again provide informed consent.

Note that if discrepancies exist between minor features of the statistical analysis as planned in the protocol, and the final SAP, a protocol amendment will not be issued, and the SAP will prevail.

13.13. Study Termination

Aeglea 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. The Investigator should notify the IRB/EC in writing of the study's completion or early termination and send a copy of the notification to Aeglea. The sponsor defines the end of study for an individual patient as completion of the final follow up visit. The end of trial is defined as the point at which all participating patients have completed the final follow up visit after study termination when study drug is available by other means.

14. ELECTRONIC CASE REPORT FORMS AND SOURCE DOCUMENTS

Electronic data capture (EDC) will be used. Therefore, patient data from source documents will be entered directly into the clinical database at the Investigator Sites using eCRFs. The Investigator is responsible for assuring that source documentation is appropriately maintained, and that data entered into the eCRF are complete and accurate, and that entries and updates are performed in a timely manner.

Aeglea personnel (or designees) will review the data entered by investigational staff for completeness and accuracy. If potential discrepancies are discovered, electronic data queries stating the nature of the potential discrepancy and requesting clarification will be sent to the investigational site via the EDC system. Designated Investigator site staff are required to respond promptly to queries and to make any necessary changes to the data.

15. STUDY MONITORING AND AUDITING

Qualified individuals designated by Aeglea BioTherapeutics, Inc. will monitor all aspects of the study according to ICH/GCP and standard operating procedures for compliance with applicable government 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 patients, 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 Aeglea or its designees. The review of the patients' medical records will be performed in a manner to ensure that patient confidentiality is adequately maintained. Further details of the study monitoring will be outlined in a Monitoring Plan.

Members of the Aeglea Quality Assurance Department or designees may conduct an audit of a clinical site at any time during or after completion of the study. The Investigator will be informed if an audit is to take place and advised as to the scope of the audit. Representatives of the FDA or other regulatory agencies may also conduct an audit of the study. If informed of such an inspection, the Investigator should notify Aeglea immediately. 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 patient confidentiality to the extent that local, state, and federal laws apply.

16. PRIVACY AND CONFIDENTIALITY

All US-based sites and laboratories or entities providing support for this study must, where applicable, comply with Health Insurance Portability and Accountability Act (HIPAA) of 1996. A site that is not a covered entity as defined by HIPAA must provide documentation of this fact to the Sponsor/CRO.

The confidentiality of records that may be able to identify patients will be protected in accordance with applicable laws, regulations, and guidelines.

After patients have consented to take part in the study, the Sponsor and/or its representatives review their medical records and data collected during the study. These records and data may, in addition, be reviewed and copied 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; and the IRB(s)/EC(s) which gave approval for the study to proceed. 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 patients' identities.

Subjects are assigned a unique identifying number; however, their initials and date of birth may also be collected and used to assist the Sponsor to verify the accuracy of the data (eg, to confirm that laboratory results have been assigned to the correct patient).

The results of studies containing patients' 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.

17. RETENTION OF RECORDS

The Investigator must retain all study records required by Aeglea BioTherapeutics, Inc. and by the applicable regulations in a secure and safe facility. The Investigator must consult an Aeglea representative before disposal of any study records and must notify Aeglea 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 (ie, 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 (eg, patient charts), as well as any original source documents that are electronic as required by applicable regulatory requirements.

All study records must be retained for at least 2 years after the last approval of a marketing application in the US or an ICH region and until (1) there are no pending or contemplated marketing applications in the US or an ICH region or (2) ≥ 2 years have elapsed since the formal discontinuation of clinical development of the IP. The Investigator/institution should retain patient identifiers for ≥ 15 years after the completion or discontinuation of the study. Patient files and other source data must be kept for the maximum period of time permitted by the hospital, institution, or private practice, but not less than 15 years. These documents should be retained for a longer period, however, if required by the applicable regulatory requirements or by an Aeglea agreement. Aeglea must be notified and will assist with retention should Investigator/institution be unable to continue maintenance of patient files for the full 15 years. It is the responsibility of Aeglea to inform the Investigator/institution as to when these documents no longer need to be retained.

18. USE OF INFORMATION AND PUBLICATION

Aeglea BioTherapeutics, Inc. recognizes the importance of communicating medical study data and therefore encourages their 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 Aeglea, 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 Aeglea. Any Investigator wishing to publish or present any study finding must present a manuscript or abstract to Aeglea 120 days prior to submission for publication or presentation to provide Aeglea an opportunity for review and comment.

19. INVESTIGATOR RESPONSIBILITIES

19.1. Conduct of Study and Protection of Human Subjects

In accordance with Form FDA 1572, the Investigator will ensure that:

- He/she will conduct the study in accordance with the relevant, current protocol and will only make changes in a protocol after notifying the Sponsor, except when necessary to protect the safety, rights, or welfare of subjects.
- He/she will personally conduct or supervise the study.
- He/she will inform any potential subjects, or any persons used as controls, that the drugs are being used for investigational purposes and he or she will ensure that the requirements relating to obtaining informed consent in 21 Part 50 and IRB review and approval in 21 CFR Part 56 are met.
- He/she will report to the Sponsor adverse experiences that occur in the course of the investigation in accordance with 21 CFR 312.64.
- He/she has read and understands the information in the IB, including potential risks and side effects of the drug.
- His/her staff and all persons who assist in the conduct of the study are informed about their obligations in meeting the above commitments.
- He/she will ensure adequate and accurate records in accordance with 21 CFR 312.62 and make those records available for inspection in accordance with 21 CFR 312.68.
- He/she will ensure that the IRB/EC complies with the requirements of 21 CFR Part 56, and other applicable regulations, and conducts initial and ongoing reviews and approvals of the study. He/she will also ensure that any change in research activity and all problems involving risks to human subjects or others are reported to the IRB/EC. Additionally, he/she will not make any changes in the research without IRB/EC approval, except where necessary to eliminate apparent immediate hazards to human subjects.
- He/she agrees to comply with all other requirements regarding the obligations of clinical Investigators and all other pertinent requirements in 21 CFR Part 312.

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Reason for signing: Approved	Name: [redacted] Role: [redacted] Date of Signature: 28-Jun-2021 17:39:49 GMT+0000

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