Official Title: A Phase 3 Multi-Center Study to Evaluate the Safety and Efficacy of Subcutaneous Injections of Pegvaliase in Adolescent Subjects (Ages 12-17) with Phenylketonuria Featuring an Open-Label Randomized Two-Arm (Active vs Diet-Only Control) Design

NCT Number: NCT05270837

Applicant/MAH: BioMarin Pharmaceutical Inc.

Version Date: 06 Nov 2023

16 APPENDICES

16.1 Study Information

16.1.1 Protocol and Protocol Amendments

Original Protocol (dated 22 Sep 2021)

Protocol Amendment 1 (dated 26 Jan 2022)

Protocol Amendment 2 (dated 23 Oct 2023)

Protocol Amendment 1 (ex-U.S) (dated 06 Jun 2022)

Protocol Amendment 2 (ex U.S) (dated 06 Nov 2023)

CLINICAL STUDY PROTOCOL

Protocol Title:	A Phase 3 Multi-Center Study to Evaluate the Safety and Efficacy of Subcutaneous Injections of Pegvaliase in Adolescent Subjects (Ages 12-17) with Phenylketonuria Featuring an Open-Label Randomized Two-Arm (Active vs Diet-Only Control) Design
Protocol Number:	165-306
Amendment Number:	Original protocol
Compound:	pegvaliase (formerly BMN 165)
Short Title:	A Phase 3 Multi-Center Study to Evaluate the Safety and Efficacy of Subcutaneous Injections of Pegvaliase in Adolescent Subjects (Ages 12-17) with Phenylketonuria
Study Phase:	Phase 3
Sponsor Name:	BioMarin Pharmaceutical Inc.
Legal Registered Address:	105 Digital Drive Novato, CA 94949
Regulatory Agency Identifier Number(s):	IND: 076269 EudraCT: 2021-005058-27
Approval Date:	22 September 2021

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May not be divulged, published, or otherwise disclosed to others without prior written approval from BioMarin.
This study will be conducted according to the principles of Good Clinical Practice as described in the U.S. Code of Federal Regulations and the International Conference on Harmonisation Guidelines, including the archiving of essential documents.

I have read this study protocol and agree to conduct this study in compliance with the protocol, all applicable regulations, guidelines, and other information supplied to me.

Investigator Signature Date

Printed name: _____

Accepted for the Sponsor:

PI PI

PI Monitor Signature Date

Printed name: PI

PI

Medical monitor contact information is available in Appendix 3, Section 10.3.

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1 PROTOCOL SUMMARY

1.1 Synopsis

Protocol Title: A Phase 3 Multi-Center Study to Evaluate the Safety and Efficacy of Subcutaneous Injections of Pegvaliase in Adolescent Subjects (Ages 12-17) with Phenylketonuria Featuring an Open-Label Randomized Two-Arm (Active vs Diet-Only Control) Design

Short Title: A Phase 3 Multi-Center Study to Evaluate the Safety and Efficacy of Subcutaneous Injections of Pegvaliase in Adolescent Subjects (Ages 12-17) with Phenylketonuria

Study Design Rationale:

This Phase 3 multi-center study is designed to evaluate the safety and efficacy of pegvaliase administered daily to adolescent subjects (ages 12-17) with phenylketonuria (PKU). Subjects will be randomized in a 2:1 ratio to the active (pegvaliase) and control (diet-only) treatment arms, respectively, with 36 subjects receiving pegvaliase and 18 subjects managing their PKU with diet alone. The primary efficacy endpoint is the change from baseline in blood phenylalanine (Phe) following 72 weeks on study (the end of Part 1). Beginning Week 73, subjects in the active (pegvaliase) treatment arm will continue to receive pegvaliase in the Extension Phase (Part 2) for up to an additional 80 weeks (through Week 153). Subjects in the control (diet-only) treatment arm will initiate pegvaliase treatment beginning Week 73 and, from Weeks 73 through 145, follow the same dosing and assessment schedule that the active subjects followed from Weeks 1 through 73, after which they will receive pegvaliase for up to 8 additional weeks (through Week 153).

A randomized control trial (RCT) design, with diet-only management of PKU as the control, will allow for robust data collection and minimize the risk of confounding factors influencing the results. An open-label diet-only control arm is the best comparator for this age group as daily placebo injections would place undue burden on adolescent PKU patients.

In Part 1 (through the Week 73 visit), randomized subjects in both the control (diet-only) and active (pegvaliase) treatment arms will be required to maintain the same level of protein intake they established during Screening/Run-in. In Part 2, subjects in the control (diet-only) arm must continue to maintain this prescribed level of protein intake, whereas subjects randomized to the active (pegvaliase) arm whose blood Phe concentration is ≤ 360 µmol/L for a minimum of 4 weeks may adjust dietary protein intake based on individual subject response to pegvaliase and guidance from the investigator. Subjects are required to maintain

a consistent level of protein intake during the study to ensure that the safety and efficacy endpoints are attributable to pegvaliase rather than to changes in dietary protein intake.

Pegvaliase will be subcutaneously injected in the clinic for the first 8 weeks and thereafter at the clinic or at home by a trained subject, caregiver, adult observer, or visiting healthcare provider. Administration at home makes daily treatment less burdensome for subjects, thereby improving treatment compliance.

An adult observer is required for a minimum of 1 hour post injection throughout the study. An adult observer is defined as someone who will be present with the subject during and after pegvaliase administration, is able to recognize the signs and symptoms of an anaphylaxis episode, and is able to call for emergency medical support and administer epinephrine, if warranted. The requirement for an adult observer is included to ensure that subjects self-administering pegvaliase would have support in recognizing and quickly responding to signs and symptoms of anaphylaxis.

The primary efficacy endpoint is the change from treatment naïve baseline in blood Phe following 72 weeks on study (the end of Part 1). Blood Phe level is the established clinical marker used to diagnose PKU and assess disease control, and reduction of blood Phe concentration to target levels is the universally acknowledged treatment goal for PKU patients.

Objectives and Endpoints:

The primary, secondary, and exploratory objectives and associated endpoints are shown below. Refer to the Schedule of Activities (SoA) for the complete listing of assessment timepoints.

Objectives	Endpoints
Primary	
To evaluate the efficacy of pegvaliase in adolescent subjects with PKU	The primary efficacy endpoint is change in blood Phe concentration from treatment naïve baseline following 72 weeks on study. Change in blood Phe from baseline is compared between subjects in the active (pegvaliase) and the control (diet-only) treatment arms.
To evaluate the safety of pegvaliase in adolescent subjects with PKU	• Adverse events (AEs), including serious AEs (SAEs) • Clinical laboratory tests (chemistry, hematology, and urinalysis) results • Vital signs • Physical examination (including height, weight) • Electrocardiogram (ECG) test results • Immunogenicity test results (anti-PEG IgG, anti-PEG IgM, anti-PAL IgG, anti-PAL IgM, TAb, NAb, and anti-pegvaliase IgE [hypersensitivity reaction visits only]).
Secondary	
To characterize total dietary protein intake in adolescent subjects with PKU after pegvaliase treatment	Total dietary protein intake must remain stable for change in blood Phe, the primary endpoint, to be accurately assessed. To gauge the stability of total protein intake, the change from baseline at Week 73 in total dietary protein intake (defined as the sum of the protein intake from medical food and intact food) will be measured.
To characterize the pharmacokinetics (PK) of pegvaliase in adolescent subjects with PKU	Trough plasma PK samples will be collected and analyzed as specified in the SoA. PK parameters assessed with intensive PK sampling will include $AUC_{0\text{-}24hr,ss}$, $C_{max,ss}$, $T_{max,ss}$, $t_{1/2}$, $CL_{ss/F}$, and $V_{dz/F}$. For subjects randomized to the active (pegvaliase) treatment arm, intensive PK sampling will be performed at Week 73 and for dose escalations to 20, 40, and 60 mg/day. For subjects randomized to the control (diet-only) treatment arm, there are no PK assessments in Part 1. In Part 2, intensive PK sampling will be performed at Week 145 and for dose escalations to 20, 40, and 60 mg/day.

Objectives	Endpoints
Exploratory Objectives	
To evaluate the effect of pegvaliase treatment on neurocognitive outcomes in adolescent subjects with PKU.	• The ADHD Rating Scale IV inattention subscale (ADHD-RS IV) rated by the parent/guardian and performed at Baseline (Day 1, pre-dose) and every 12 weeks throughout the study. • The Behavior Rating Inventory of Executive Function (BRIEF) rated by the parent/guardian and performed at Baseline (Day 1, pre-dose) and every 12 weeks throughout the study.
Explore the biochemical, molecular, and cellular aspects of PKU.	Blood will be collected to evaluate biochemical, molecular, and cellular aspects of PKU and to develop the assays used for these evaluations.
PAH genotyping	Testing will be performed on samples collected Day 1 pre-dose where allowed by local health authorities and the EC/IRB.

Overall Design:

Study 165-306 is an open-label, two-arm, randomized control evaluation of pegvaliase in approximately 54 subjects 12-17 years old, inclusive, with diet only as the control arm. To be eligible for the study, potential subjects must meet all eligibility requirements described in the protocol, including the following selected key inclusion criteria:

- Is 12 to 17 years old, inclusive, at the start of the Screening/Run-in Period (Day -28).
- Diagnosis of PKU and failure to maintain recommended blood Phe levels on existing management (sapropterin dihydrochloride and/or Phe-restricted diet) demonstrated by 2 blood Phe concentration measurements > 600 µmol/L during the Screening/Run-in Period (7 to 10 days between blood Phe assessments) and average blood Phe concentration > 600 µmol/L over the past 12 months (per available data).
- Willing and able to maintain and adjust dietary and medical protein food intake according to the study protocol.
- An adult (≥ 18 years of age) has been identified who is willing and competent to observe the subject during study drug administration and for a minimum of 1 hour following administration. A home healthcare nurse may perform the study drug observations consistent with local regulatory and site requirements.

Selected key exclusion criteria include the following:

- Potential subjects are not eligible if they are unable to identify and/or communicate to others that they are experiencing symptoms of potential anaphylaxis due to cognitive impairment or other reasons.

Following enrollment, subjects will be randomized 2:1 to the active (pegvaliase) and control (diet-only) treatment arms, with 36 subjects receiving daily pegvaliase and 18 subjects managing PKU by diet alone. Treatment assignment will be stratified by baseline blood Phe (average of the 3 pre-treatment blood Phe assessments performed through Day 1 pre-dose) of ≤ 1000 µmol/L or > 1000 µmol/L. The primary efficacy endpoint is the change from baseline blood Phe following 72 weeks on study (the end of Part 1). Subjects in the active and control arms will follow the same visit schedules and perform the same assessments throughout the 72-week Primary Treatment Phase (Part 1) except that the control subjects will not be receiving pegvaliase so will not have PK draws or immunogenicity assessments.

After providing informed consent, subjects will enter the Screening/Run-in period, which will last 4 weeks and include 2 assessments of blood Phe levels, 7 to 10 days apart, to determine eligibility. Following confirmation that the 2 blood Phe results are > 600 µmol/L and all other eligibility criteria have been met, subjects may be enrolled in the study on Day 1. During the Screening/Run-in and the Primary Treatment Phase (Part 1), subjects will be required to maintain stable protein intake from both medical food and/or intact food.

The duration of Part 1 is 72 weeks, during which pegvaliase treatment will be initiated for subjects in the active arm using an Induction/Titration/Maintenance (I/T/M) dosing regimen. The I/T/M dosing regimen consists of Induction/Titration Dosing (9 weeks) and Maintenance Dosing. Required clinic visits during Part 1 will occur weekly for the first 8 weeks, every 4 weeks thereafter, and when the pegvaliase dose is escalated to 20, 40, or 60 mg/day. The assessments performed at the clinic visits include measurement of blood Phe, neurocognitive assessments, PK blood draws (active subjects only), and safety assessments.

Beginning Week 73, subjects in the active treatment arm will continue to receive pegvaliase in the Extension Phase (Part 2) for up to 80 additional weeks (through Week 153). Beginning Week 73, subjects in the control treatment arm will initiate pegvaliase treatment and follow a parallel schedule for dosing, study visits, and assessments in Weeks 73 through 145 to that followed by the active arm subjects during the Primary Treatment Phase (Part 1) from Weeks 1 through 73, after which they will receive pegvaliase for up to 8 additional weeks (through Week 153).

Brief Summary:

The purpose of Study 165-306 is to evaluate the safety and efficacy of pegvaliase and characterize the PK of pegvaliase in adolescent subjects with PKU. Study details include:

Condition/Disease: PKU is a rare autosomal recessive genetic disorder caused by mutations in the phenylalanine hydroxylase (PAH) gene, leading to an absence or deficiency in PAH

enzyme activity and subsequent elevation of the amino acid phenylalanine (Phe) in the blood, known as hyperphenylalaninemia (HPA) (Vockley 2014; Mitchell 2011). Deficiency of PAH results in an abnormally elevated concentration of Phe, which is toxic to the brain. High Phe levels during infancy and early childhood cause profound neurocognitive and developmental defects, and poorly controlled blood Phe levels in older children and adults are associated with learning disabilities, attention deficit hyperactivity disorder, behavioral problems, and psychiatric symptoms.

Study Hypothesis: The study hypothesis is that subjects receiving pegvaliase will show a greater reduction in blood Phe following 72 weeks on study compared to subjects in the diet-only control group.

Study Duration: The study duration is up to 160 weeks for subjects randomized to active (pegvaliase) treatment. This includes a 4-week Screening/Run-in period, up to 152 weeks of pegvaliase treatment in Parts 1 and 2, and the Study Completion/Early Termination (SC/ET) visit 4 weeks after the final dose of pegvaliase. The study duration is also 160 weeks for subjects randomized to control (diet-only) treatment. This includes a 4-week Screening/Run-in period, 72 weeks managing PKU with diet alone while following the Part 1 assessment schedule, 72 weeks of pegvaliase treatment following the same Part 1 assessment schedule, 8 additional weeks of pegvaliase treatment, and the SC/ET visit 4 weeks after the final dose of pegvaliase.

Treatment Duration: The pegvaliase treatment duration will be 152 weeks for subjects randomized to the active (pegvaliase) treatment arm (n=36). The pegvaliase treatment duration will be 80 weeks for subjects randomized to the control (diet-only) treatment arm (n=18).

Health Measurement/Observation: Reduction in blood Phe.

Visit Frequency: For both active and control subjects, clinic visits in Part 1 will occur weekly for the first 8 weeks, then every 4 weeks through Week 73. For subjects randomized to active (pegvaliase) treatment, clinic visits in Part 2 will occur every 4 weeks through Week 153. For subjects randomized to control (diet-only) treatment, clinic visits in Part 2 will occur weekly for the first 8 weeks, then every 4 weeks through Week 153. For subjects randomized to active (pegvaliase) treatment in Part 1 and subjects randomized to control (diet-only) treatment in Part 2, clinic visits will also occur when their dose level is escalated to 20, 40, and 60 mg/day.

Number of Subjects:

Approximately 54 subjects will be enrolled and randomized to the pegvaliase or diet-only groups in a 2:1 ratio. Assuming the mean (SD) reduction in Phe following 72 weeks on study is 640 (570) µmol/L in the pegvaliase group and 100 (250) µmol/L in the diet-only group, a total sample size of 54 subjects will provide more than 90% power to detect a treatment difference, based on the two-sample T-test with unequal variance and a significance level of 0.05 (two sided). The assumption of treatment effect is based on clinical data from previous pegvaliase studies and a PKU registry study. See the Statistical Analysis Plan (SAP) for additional details on the sample size calculation.

Approximately 72 subjects will be screened for study participation such that approximately 54 subjects will be enrolled in the study.

Note: Potential subjects who are screened for the purpose of determining eligibility for the study, but do not participate in the study, are not considered enrolled.

Treatment Groups and Duration:

The duration of the Primary Treatment Phase (Part 1) is 72 weeks, during which pegvaliase treatment will be initiated for subjects randomized to the active (pegvaliase) treatment arm using an I/T/M dosing regimen. Induction will consist of a 4-week period when subjects randomized to active drug will receive pegvaliase subcutaneously with a pre-filled syringe (PFS) at a fixed dose of 2.5 mg/week and during which the dosing regimen will not be modified in response to blood Phe concentration. Once subjects complete induction they will titrate up to a dose of 10 mg/day. The dosing frequency will be increased gradually during titration dosing to daily (7 days/week). During Maintenance Dosing the dose will be increased to 20 mg/day, 40 mg/day, and 60 mg/day depending upon individual blood Phe lowering efficacy (per the investigator's discretion). The dose should be increased to 40 mg/day if blood Phe is > 360 µmol/L after 24 weeks dosing at 20 mg/day and increased to 60 mg/day if blood Phe is > 360 µmol/L after 16 weeks dosing at 40 mg/day. BioMarin recommends that the target blood Phe levels should be based on individual needs, with a target blood Phe ≤ 600 µmol/L or lower. During the Primary Treatment Phase (Part 1), subjects randomized to the control (diet-only) treatment arm will have their PKU managed by diet alone.

Following completion of Part 1, subjects randomized to active (pegvaliase) treatment will enter the Extension Phase (Part 2), during which subjects will continue dosing with pegvaliase at their optimal maintenance dose level for up to an additional 80 weeks (through Week 153) in order to evaluate the long-term safety and efficacy of pegvaliase in adolescent

subjects with PKU. Following completion of Part 1, subjects randomized to control (diet-only) treatment will repeat the Part 1 assessment schedule while initiating pegvaliase dosing from Weeks 73 through 145, after which they will receive pegvaliase for up to 8 additional weeks (through Week 153).

Data Monitoring Committee:

An independent Data Monitoring Committee (DMC) will monitor the safety of study subjects. The DMC is an independent committee that acts in an advisory capacity to BioMarin.

1.2 Study Schema

Figure 1.2.1: Active (Pegvaliase) vs Control (Diet-Only) Treatment Arms (Randomized Treatment Assignment)

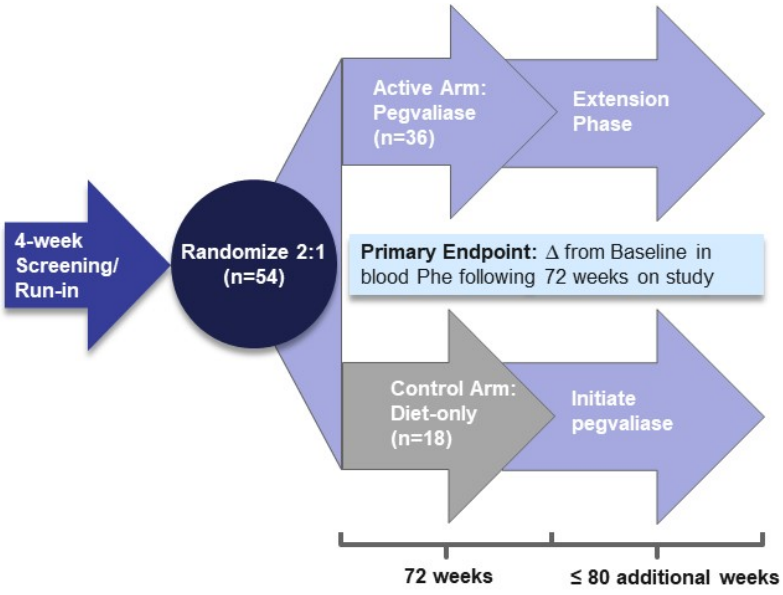
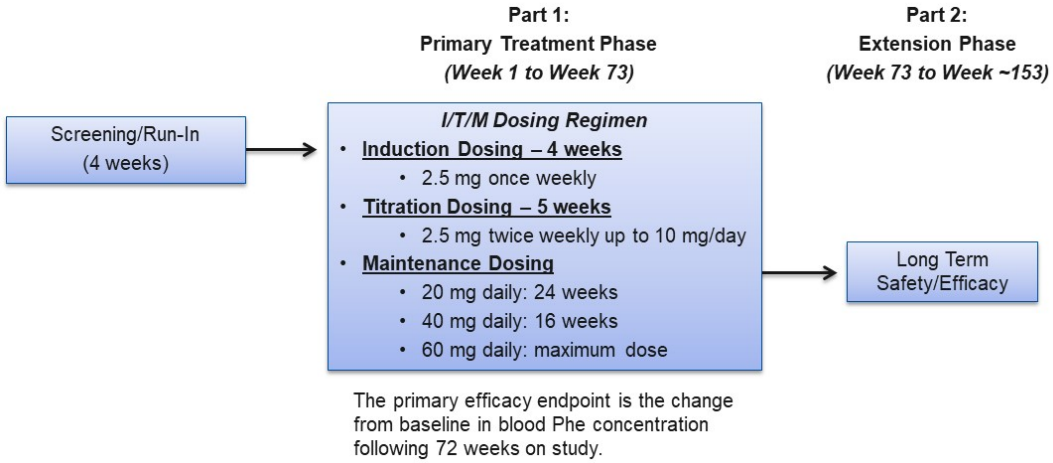


Figure 1.2.2: Dosing Schematic for Active (Pegvaliase) Treatment Arm



Note: The 18 control (diet-only) subjects will follow the Part 1 assessment schedule (except for pegvaliase dosing and PK/immunogenicity assessments) from Weeks 1 through Week 73 and then repeat the Part 1 assessment schedule including pegvaliase dosing and PK/immunogenicity assessments from Week 73 through 145, after which they will receive pegvaliase for 8 additional weeks (through Week 153).

Prior to dose escalation, the minimum amount of time pegvaliase can be dosed at 20 mg/day before escalating is 24 weeks and the minimum amount of time at 40 mg/day before escalating is 16 weeks. The dose should be increased to 40 mg/day if blood Phe is > 360 µmol/L after 24 weeks dosing at 20 mg/day, and increased to 60 mg/day if blood Phe is > 360 µmol/L after 16 weeks dosing at 40 mg/day.

1.3 Schedule of Activities (SoA)

Table 1.3.1: Schedule of Activities for Part 1 (Subjects Randomized to Pegvaliase Treatment Arm)

Assessment [a]	Study Week	Screen[b]	1	2	3	4	5	6	7	8	9	13	17	21	25	29	33	37	41	45	49	53	57	61	65	69	73	HRV[c]	SC/ET[d]
	Study Day	-28/1	1	8	15	22	29	36	43	50	57	85	113	141	169	197	225	253	281	309	337	365	393	421	449	477	505		
	Window (Days)			±3	±3	±3	±3	±3	±3	±3	±3	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7		
Informed consent		X																											
Drug administration training [e]			X																										
Study drug observer training [f]		X																											
Demographics		X																											
Medical history [g]		X																											
HIV, hepatitis B and C screens [h]		X																											
PAH genotyping [i]			X																										
12 lead ECG		X	X																										X
Physical examination [j]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Weight		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Height		X	X				X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
Vital signs		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Clinical safety laboratory tests [k]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
C-reactive protein		X	X								X		X		X		X		X		X		X		X		X	X	X
Erythrocyte sedimentation rate [l]		X	X																										X
Serum cortisol [m]			X												X						X						X		X
Complements C3 and C4			X				X				X		X		X		X		X		X		X		X		X	X	X
Tryptase			X																									X	
Urine albumin/creatinine ratio [n]			X								X		X		X		X		X		X		X		X		X	X	X
Urine pregnancy test [o]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
3-day diet diary [p]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
PK (plasma pegvaliase) [q]			X				X				X	X	X	X	X		X		X		X		X		X		X		X

Assessment [a]	Study Week	Screen[b]	1	2	3	4	5	6	7	8	9	13	17	21	25	29	33	37	41	45	49	53	57	61	65	69	73	HRV[c]	SC/ET[d]
	Study Day	-28/1	1	8	15	22	29	36	43	50	57	85	113	141	169	197	225	253	281	309	337	365	393	421	449	477	505		
	Window (Days)			±3	±3	±3	±3	±3	±3	±3	±3	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7		
Blood for exploratory analyses [i]			X				X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
Blood Phe, tyrosine [r]		X [b]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
Immunogenicity assessments [s]			X	X	X	X	X	X	X	X	X	X	X	X	X		X		X		X		X		X		X	X	X
ADHD-RS IV [t]			X									X			X			X			X			X			X		X
BRIEF [u]			X									X			X			X			X			X			X		X
Weekly call (non-visit weeks) [v]																													
Adverse events [w]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant medication [w]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Administer study drug [x]			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		

ACTH, adrenocorticotropic hormone; ADHD-RS IV, attention deficit hyperactivity disorder rating scale IV; BRIEF, Behavior Rating Inventory of Executive Function; EC/IRB, Ethics Committee/Institutional Review Board; ECG, electrocardiogram; eCRF, electronic Case Report Form; HRV, hypersensitivity reaction visit; NSAID, non-steroidal anti-inflammatory drug; PAH, phenylalanine hydroxylase; PE, physical examination; PK, pharmacokinetics; PKU, phenylketonuria; SC/ET, Study Completion/Early Termination visit.

[a] Scheduled visits take place at the clinic or, with the approval of the investigator or designee, virtually. For virtual visits the investigator/designee performs assessments while online with the subject, apart from specimen collections and vital signs, which are performed by home healthcare personnel. Non-clinic visits are via telephone. Assessments are performed pre-dose unless otherwise specified.

[b] After written informed consent, screening assessments must be performed within 28 days prior to Part 1, Day 1. Subjects will be assessed for blood Phe concentration during Screening/Run-in with 2 measurements 7 to 10 days apart. These 2 blood Phe measurements should be scheduled to occur: (1) in the Day -25 to Day -23 window; and (2) 7 to 10 days later (ie, in the Day -18 to Day -13 window).

[c] If the investigator determines that the NCI-CTCAE Grade ≥ 3 hypersensitivity reaction is related to administration with study drug, the subject will be asked to return to the clinic within 24 hours of event onset for a hypersensitivity reaction visit (HRV). Sampling for anti-pegvaliase IgE should be performed 8 to 24 hours after event onset and before the next dose of study drug.

[d] Subjects should perform the Study Completion (SC) Visit 4 weeks after the last dose of study drug or after completion of study participation, whichever occurs last. For subjects who terminate from the study early, the Early Termination (ET) Visit occurs within 4 weeks of the last study drug dose or after withdrawal from study participation, whichever occurs last. Subjects who discontinue from study drug early will be asked to continue to complete the remaining study assessments until study completion as long as such continued participation will not detrimentally affect the health, safety, and welfare of the subject per investigator determination.

[e] Once subject/caregiver competence is documented, the subject/caregiver administers study drug daily.

[f] Must be completed prior to administration of the first dose of study drug. Additional observers identified after screening must be trained.

[g] PKU history includes highest blood Phe (and age), and subject appraisal of metabolic control.

[h] Subjects with documented negative results within the last 4 weeks do not need to be retested.

[i] Sample collection should be performed where allowed by country-specific regulations and EC/IRB.

[j] A full physical examination (PE) will be performed at Screening, Baseline, Week 73, and the SC/ET visit. A brief PE will be performed at other visits. A full PE will include, at a minimum, general appearance (head, eyes, ears, nose, and throat) and an assessment of cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, musculoskeletal, and neurologic systems. A brief PE may be performed at the discretion of the investigator based on the subject's clinical condition and may include, but is not limited to, general appearance and an assessment of cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, musculoskeletal, and neurologic systems.

[k] It is recommended that urine samples are obtained as a first or second morning void. In the event of elevated urinary protein on a test result, a repeat urinalysis should be performed. This repeat urine sample must be performed in the morning at the first or second morning void to allow for accurate test results and may be performed by a home healthcare nurse.

[l] To be done by a local laboratory.

[m] Serum cortisol samples should be taken before study drug administration in the morning before 10:00 am. A home healthcare nurse may collect samples for subject convenience. If 2 results are low and abnormal, the subject will be asked to perform additional sampling for plasma ACTH and a low-dose conventional ACTH stimulation test or the ACTH test method based on the clinical practice at the site.

[n] It is recommended that urine samples be obtained as a first or second morning void. Subjects with a confirmed urine/albumin creatinine ratio of ≥ 100 mg/g should be referred to a nephrologist for consultation if results were within normal range at Baseline. Subjects who had elevated results at Baseline followed by a confirmed subsequent increase of 100-200 mg/g from Baseline should also be referred to a nephrologist for consultation.

[o] To be done locally (if applicable). If urine pregnancy test is positive or equivocal, serum pregnancy test (central laboratory) must be done.

[p] Subjects should record at home all food, beverages, special low-protein foods, and medical foods consumed during the 3 consecutive days preceding a study visit. No change in protein (dietary and medical food) intake is allowed in Part 1. During Screening/Run-in, 3-day diet diary assessments are performed 3 times, each time covering the 3-day periods prior to blood Phe assessments (and collected on the day of those assessments). The 3 Screening/Run-in blood Phe assessments are scheduled to occur (1) at Day -25 to Day -23; (2) 7 to 10 days later (ie, in the Day -18 to Day -13 window); and (3) at Day 1 pre-dose. Thus, the 3 day diet assessments will assess diet for (1) the 3 days within the Day -28 to Day -23 window immediately prior to the first blood Phe assessment; (2) the 3 days within the Day -21 to Day -15 window immediately prior to the second blood Phe assessment; and (3) for Day -3 to Day 1.

[q] Blood is collected before study drug administration (pre-dose). Intensive PK sampling will be performed at Week 73 and at dose escalation visits in all subjects. Samples will be taken at pre-dose, 2, 4, 8, 12, and 24 hours post dose. The 24-hour sample will be taken prior to the next daily dose.

[r] Blood is collected for blood Phe analysis after fasting a minimum of 2.5 hours. In addition to the blood draws for blood Phe analysis collected in the clinic and shown in the SoA, there are 5 required blood collections that may be drawn either at the clinic or elsewhere (eg, at home). These are to be collected at Weeks 11, 15, and 19, and 2 weeks prior to the planned escalations to 40 and 60 mg/day.

[s] Immunogenicity assays include total anti-pegvaliase antibodies (TAb), anti-PAL IgG, anti-PAL IgM, anti-PEG IgM, anti-PEG IgG, and neutralizing antibodies (NAb). At the HRV, only anti-pegvaliase IgE will be assessed.

[t] Completed by parent/guardian.

[u] Completed by parent/guardian.

[v] Between scheduled clinic visits, the clinic staff will contact the subject weekly to monitor if the subject is experiencing problems with drug administration, to ask about any AEs or concomitant medications, and to answer questions.

[w] AEs and concomitant medications should be noted whenever a subject is assessed by study personnel. Following signed informed consent and prior to the first dose of pegvaliase, only SAEs associated with study procedures are collected. After the first dose all AEs and SAEs are collected until 4 weeks after the last study drug dose or the Early Termination Visit, whichever occurs last. If there is a severe (Grade 3) injection site reaction, the subject may be referred to a dermatologist for consultation and a skin biopsy (optional; if clinically indicated). If photographs of the severe injection site reaction are taken, they may be collected by the sponsor.

[x] Subjects must be premedicated with an H1 antagonist, and an H2 antagonist, and an antipyretic approximately 2 to 3 hours prior to each dose of study drug until they reach the Maintenance Phase, defined as when subjects achieve blood Phe ≤ 600 µmol/L while they are on a stable dose of pegvaliase. Premedication may be considered during the Maintenance Phase at the investigator's discretion, eg, approximately 2 to 3 hours prior to study drug for 1 week upon reintroduction of study drug, upon resolution of an AE, following any dose interruption of ≥ 4 days, and for any dose increases in the Extension Phase. Subjects may also be premedicated at any other time in the study per investigator discretion. When an NSAID is administered as a premedication, it should be given with food. For non-clinic visits, subjects are asked about study drug administration.

Table 1.3.2: Schedule of Activities for Part 2 (Subjects Randomized to Pegvaliase Treatment Arm)

Assessment [a]	Study Week	73[b]	77	81	85	89	93	97	101	105	109	113	117	121	125	129	133	137	141	145	149	153	HRV[c]	SC/ET[d]
	Study Day	505	533	561	589	617	645	673	701	729	757	785	813	841	869	897	925	953	981	1009	1037	1065		
	Window (Days)	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7		
Study drug observer training [e]																								
12 lead ECG																								X
Physical examination [f]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Weight	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Height	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
Vital signs	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Clinical safety laboratory tests [g]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
C-reactive protein	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Erythrocyte sedimentation rate [h]																								X
Serum cortisol [i]	X						X						X								X			X
Complements C3 and C4	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Tryptase																							X	
Urine albumin/creatinine ratio [j]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Urine pregnancy test [k]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
3-day diet diary [l]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
PK (plasma pegvaliase) [m]	X		X		X		X		X		X		X		X		X		X		X			X
Blood for exploratory analyses [n]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
Blood Phe, tyrosine [o]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Immunogenicity assessments [p]	X		X		X		X		X		X		X		X		X		X		X	X	X	X
ADHD-RS IV [q]	X			X			X			X			X			X			X					X
BRIEF [r]	X			X			X			X			X			X			X					X
Weekly call (non-visit weeks) [s]																								
Adverse events [t]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant medication [t]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

Assessment [a]	Study Week	73[b]	77	81	85	89	93	97	101	105	109	113	117	121	125	129	133	137	141	145	149	153	HRV[c]	SC/ET[d]
	Study Day	505	533	561	589	617	645	673	701	729	757	785	813	841	869	897	925	953	981	1009	1037	1065		
	Window (Days)	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7		
Administer study drug [u]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		

ACTH, adrenocorticotropic hormone; ADHD-RS IV, attention deficit hyperactivity disorder rating scale IV; BRIEF, Behavior Rating Inventory of Executive Function; ECG, electrocardiogram; eCRF, electronic Case Report Form; HRV, hypersensitivity reaction visit; PE, physical examination; PK, pharmacokinetics; PKU, phenylketonuria; NSAID, non-steroidal anti-inflammatory drug; SC/ET, Study Completion/Early Termination visit.

[a] Scheduled visits take place at the clinic or, with the approval of the investigator or designee, virtually. For virtual visits the investigator/designee performs assessments while online with the subject, apart from specimen collections and vital signs, which are performed by home healthcare personnel. Non-clinic visits are via telephone. Assessments are performed pre-dose unless otherwise specified.

[b] The Week 73 visit marks both the end of the Primary Treatment Phase (Part 1) and the beginning of the Extension Phase (Part 2). The pre-dose assessments constituting the end of the Primary Treatment Phase and the administration of study drug constituting the beginning of the Extension Phase are shown in the Week 73 columns of both Table 1.3.1 and Table 1.3.2.

[c] If the investigator determines that the NCI-CTCAE Grade ≥ 3 hypersensitivity reaction is related to administration with study drug, the subject will be asked to return to the clinic within 24 hours of event onset for a hypersensitivity reaction visit (HRV). Sampling for anti-pegvaliase IgE should be performed 8 to 24 hours after event onset and before the next dose of study drug.

[d] Subjects should perform the Study Completion (SC) Visit 4 weeks after the last dose of study drug or after completion of study participation, whichever occurs last. For subjects who terminate from the study early, the Early Termination (ET) Visit occurs within 4 weeks of the last study drug dose or after withdrawal from study participation, whichever occurs last. Subjects who discontinue from study drug early will be asked to continue to complete the remaining study assessments until study completion as long as such continued participation will not detrimentally affect the health, safety, and welfare of the subject per investigator determination.

[e] Additional observers identified after screening must be trained.

[f] A full PE will be performed at Week 73 and the SC/ET visit. A brief PE will be performed at other visits. A full PE will include, at a minimum, general appearance (head, eyes, ears, nose, and throat) and an assessment of cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, musculoskeletal, and neurologic systems. A brief PE may be performed at the discretion of the investigator based on the subject's clinical condition and may include, but is not limited to, general appearance and an assessment of cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, musculoskeletal, and neurologic systems.

[g] It is recommended that urine samples are obtained as a first or second morning void. In the event of elevated urinary protein on a test result, a repeat urinalysis should be performed. This repeat urine sample must be performed in the morning at the first or second morning void to allow for accurate test results and may be performed by a home healthcare nurse.

[h] To be done by a local laboratory.

[i] Serum cortisol samples should be taken before study drug administration in the morning before 10:00 am. A home healthcare nurse may collect samples for subject convenience. If 2 results are low and abnormal, the subject will be asked to perform additional sampling for plasma ACTH and a low-dose conventional ACTH stimulation test or the ACTH test method based on the clinical practice at the site.

[j] It is recommended that urine samples be obtained as a first or second morning void. Subjects with a confirmed urine/albumin creatinine ratio of ≥ 100 mg/g should be referred to a nephrologist for consultation if results were within normal range at Baseline. Subjects who had elevated results at Baseline followed by a confirmed subsequent increase of 100-200 mg/g from Baseline should also be referred to a nephrologist for consultation.

[k] To be done locally (if applicable). If urine pregnancy test is positive or equivocal, serum pregnancy test (central laboratory) must be done.

[l] Subjects should record at home all food, beverages, special low-protein foods, and medical foods consumed during the 3 consecutive days preceding a study visit. No change in protein (dietary and medical food) intake is allowed in Part 1.

[m] Blood is collected before study drug administration (pre-dose). Intensive PK sampling will be performed at Week 73 and at dose escalation visits in all subjects. Samples will be taken at pre-dose, 2, 4, 8, 12, and 24 hours post dose. The 24-hour sample will be taken prior to the next daily dose.

[n] Sample collection should be performed where allowed by country-specific regulations and EC/IRB.

[o] Blood is collected for blood Phe analysis after fasting a minimum of 2.5 hours.

[p] Immunogenicity assays include total anti-pegvaliase antibodies (TAb), anti-PAL IgG, anti-PAL IgM, anti-PEG IgM, anti-PEG IgG, and neutralizing antibodies (NAb). At the HRV, only anti-pegvaliase IgE will be assessed.

[q] Completed by parent/guardian.

[r] Completed by parent/guardian.

[s] Between scheduled clinic visits, the clinic staff will contact the subject weekly to monitor if the subject is experiencing problems with drug administration, to ask about any AEs or concomitant medications, and to answer questions.

[t] AEs and concomitant medications should be noted whenever a subject is assessed by study personnel. All AEs and SAEs are collected until 4 weeks after the last study drug dose or the Study Completion Visit/Early Termination visit, whichever occurs last. If there is a severe (Grade 3) injection site reaction, the subject may be referred to a dermatologist for consultation and a skin biopsy (optional; if clinically indicated). If photographs of the severe injection site reaction are taken, they may be collected by the sponsor.

[u] Subjects must be premedicated with an H1 antagonist, and an H2 antagonist, and an antipyretic approximately 2 to 3 hours prior to each dose of study drug until they reach the Maintenance Phase, defined as when subjects achieve blood Phe ≤ 600 µmol/L while they are on a stable dose of pegvaliase. Premedication may be considered during the Maintenance Phase at the investigator's discretion, eg, approximately 2 to 3 hours prior to study drug for 1 week upon reintroduction of study drug, upon resolution of an AE, following any dose interruption of ≥ 4 days, and for any dose increases in the Extension Phase. Subjects may also be premedicated at any other time in the study per investigator discretion. When an NSAID is administered as a premedication, it should be given with food. For non-clinic visits, subjects are asked about study drug administration.

Table 1.3.3: Schedule of Activities for Part 1 (Subjects Randomized to Diet-Only Treatment Arm)

Assessment [a]	Study Week	Screen[b]	1	2	3	4	5	6	7	8	9	13	17	21	25	29	33	37	41	45	49	53	57	61	65	69	73	SC/ET[c]
	Study Day	-28/1	1	8	15	22	29	36	43	50	57	85	113	141	169	197	225	253	281	309	337	365	393	421	449	477	505	
	Window (Days)			±3	±3	±3	±3	±3	±3	±3	±3	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	
Informed consent		X																										
Drug administration training [d]																											X	
Study drug observer training [e]																											X	
Demographics		X																										
Medical history (allergy, PKU) [f]		X																										
HIV, hepatitis B and C screens [g]		X																										
PAH genotyping [h]			X																									
12 lead ECG		X	X																								X	X
Physical examination [i]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Weight		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Height		X	X				X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital signs		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Clinical safety laboratory tests [j]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
C-reactive protein		X	X								X		X		X		X		X		X		X		X		X	X
Erythrocyte sedimentation rate [k]		X	X																								X	X
Serum cortisol [l]			X												X						X						X	X
Complements C3 and C4			X				X				X		X		X		X		X		X		X		X		X	X
Tryptase			X																									
Urine albumin/creatinine ratio [m]			X								X		X		X		X		X		X		X		X		X	X
Urine pregnancy test [n]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
3-day diet diary [o]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Blood for exploratory analyses [h]			X				X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Blood Phe, tyrosine [p]		X [b]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
ADHD-RS IV [q]			X									X			X			X			X			X			X	X

Assessment [a]	Study Week	Screen[b]	1	2	3	4	5	6	7	8	9	13	17	21	25	29	33	37	41	45	49	53	57	61	65	69	73	SC/ET[c]
	Study Day	-28/1	1	8	15	22	29	36	43	50	57	85	113	141	169	197	225	253	281	309	337	365	393	421	449	477	505	
	Window (Days)			±3	±3	±3	±3	±3	±3	±3	±3	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	
BRIEF [r]			X									X			X			X			X			X			X	X
Weekly call (non-visit weeks) [s]																												
Adverse events [t]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant medication [t]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

ACTH, adrenocorticotropic hormone; ADHD-RS IV, attention deficit hyperactivity disorder rating scale IV; BRIEF, Behavior Rating Inventory of Executive Function; EC/IRB, Ethics Committee/Institutional Review Board; ECG, electrocardiogram; eCRF, electronic Case Report Form; HRV, hypersensitivity reaction visit; PAH, phenylalanine hydroxylase; PE, physical examination; PK, pharmacokinetics; PKU, phenylketonuria; SC/ET, Study Completion/Early Termination visit.

[a] Scheduled visits take place at the clinic or, with the approval of the investigator or designee, virtually. For virtual visits the investigator/designee performs assessments while online with the subject, apart from specimen collections and vital signs, which are performed by home healthcare personnel. Non-clinic visits are via telephone.

[b] After written informed consent, screening assessments must be performed within 28 days prior to Part 1, Day 1. Subjects will be assessed for blood Phe concentration during Screening/Run-in with 2 measurements 7 to 10 days apart. These 2 blood Phe measurements should be scheduled to occur: (1) in the Day -25 to Day -23 window; and (2) 7 to 10 days later (ie, in the Day -18 to Day -13 window).

[c] Subjects should perform the Study Completion (SC) Visit 4 weeks after the last dose of study drug or after completion of study participation, whichever occurs last. For subjects who terminate from the study early, the Early Termination (ET) Visit occurs within 4 weeks of the last study drug dose or after withdrawal from study participation, whichever occurs last. Subjects who discontinue from study drug early will be asked to continue to complete the remaining study assessments until study completion as long as such continued participation will not detrimentally affect the health, safety, and welfare of the subject per investigator determination.

[d] Once subject/caregiver competence is documented, the subject/caregiver administers study drug daily.

[e] Must be completed prior to administration of the first dose of study drug in Part 2. Additional observers identified after Screening must be trained.

[f] PKU history includes highest blood Phe (and age), and subject appraisal of metabolic control.

[g] Subjects with documented negative results within the last 4 weeks do not need to be retested.

[h] Sample collection should be performed where allowed by country-specific regulations and EC/IRB.

[i] A full PE will be performed at Screening, Baseline, Week 73, and the SC/ET visit. A brief PE will be performed at other visits. A full PE will include, at a minimum, general appearance (head, eyes, ears, nose, and throat), and an assessment of cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, musculoskeletal, and neurologic systems. A brief PE may be performed at the discretion of the investigator based on the subject's clinical condition and may include, but is not limited to, general appearance and an assessment of cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, musculoskeletal, and neurologic systems.

[j] It is recommended that urine samples are obtained as a first or second morning void. In the event of elevated urinary protein on a test result, a repeat urinalysis should be performed. This repeat urine sample must be performed in the morning at the first or second morning void to allow for accurate test results and may be performed by a home healthcare nurse.

[k] To be done by a local laboratory.

[l] Serum cortisol samples should be taken before study drug administration in the morning before 10:00 am. A home healthcare nurse may collect samples for subject convenience. If 2 results are low and abnormal, the subject will be asked to perform additional sampling for plasma ACTH and a low-dose conventional ACTH stimulation test or the ACTH test method based on the clinical practice at the site.

[m] It is recommended that urine samples be obtained as a first or second morning void. Subjects with a confirmed urine/albumin creatinine ratio of ≥ 100 mg/g should be referred to a nephrologist for consultation if results were within normal range at Baseline. Subjects who had elevated results at Baseline followed by a confirmed subsequent increase of 100-200 mg/g from Baseline should also be referred to a nephrologist for consultation.

[n] To be done locally (if applicable). If urine pregnancy test is positive or equivocal, serum pregnancy test (central laboratory) must be done.

[o] Subjects should record at home all food, beverages, special low-protein foods, and medical foods consumed during the 3 consecutive days preceding a study visit. No change in protein (dietary and medical food) intake is allowed in Part 1. During Screening/Run-in, 3-day diet diary assessments are performed 3 times, each time covering the 3-day periods prior to blood Phe assessments (and collected on the day of those assessments). The 3 Screening/Run-in blood Phe assessments are scheduled to occur (1) at Day -25 to Day -23; (2) 7 to 10 days later (ie, in the Day -18 to Day -13 window); and (3) at Day 1 pre-dose. Thus, the 3 day diet assessments will assess diet for (1) the 3 days within the Day -28 to Day -23 window immediately prior to the first blood Phe assessment; (2) the 3 days within the Day -21 to Day -15 window immediately prior to the second blood Phe assessment; and (3) for Day -3 to Day 1.

[p] Blood is collected for blood Phe analysis after fasting a minimum of 2.5 hours. In addition to the blood draws for blood Phe analysis collected in the clinic and shown in the SoA, there are 3 required blood collections that may be drawn either at the clinic or elsewhere (eg, at home). These are to be collected at Weeks 11, 15, and 19.

[q] Completed by parent/guardian.

[r] Completed by parent/guardian.

[s] Between scheduled clinic visits, the clinic staff will contact the subject weekly to ask about any AEs or concomitant medications, and to answer questions.

[t] AEs and concomitant medications should be noted whenever a subject is assessed by study personnel. Following signed informed consent all AEs and SAEs are collected throughout Part 1 or until the Early Termination Visit, whichever occurs last.

Table 1.3.4: Schedule of Activities for Part 2 (Pegvaliase Treatment for Subjects Randomized to Diet-Only Treatment Arm)

	Study Week	73[b]	74	75	76	77	78	79	80	81	85	89	93	97	101	105	109	113	117	121	125	129	133	137	141	145	149	153		
	Study Day	505	512	519	526	533	540	547	554	561	589	617	645	673	701	729	757	785	813	841	869	897	925	953	981	1009	1037	1065	HRV[c]	SC/ET[d]
Assessment [a]	Window (Days)	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7		
Drug administration training [e]		X																												
Study drug observer training [f]		X																												
12 lead ECG		X																												X
Physical examination [g]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Weight		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Height		X				X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
Vital signs		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Clinical safety laboratory tests [h]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
C-reactive protein		X								X		X		X		X		X		X		X		X		X		X	X	X
Erythrocyte sedimentation rate [i]		X																												X
Serum cortisol [j]		X												X						X						X				X
Complements C3 and C4		X				X				X	X	X	X	X		X		X		X		X		X		X		X	X	X
Tryptase		X																											X	
Urine albumin/creatinine ratio [k]		X								X		X		X		X		X		X		X		X		X		X	X	X
Urine pregnancy test [l]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
3-day diet diary [m]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
PK (plasma pegvaliase) [n]		X				X				X	X	X	X	X		X		X		X		X		X		X		X		X
Blood for exploratory analyses [o]		X				X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
Blood Phe, tyrosine [p]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
Immunogenicity assessments [q]		X	X	X	X	X	X	X	X	X	X	X	X	X		X		X		X		X		X		X		X	X	X
ADHD-RS IV [r]		X									X			X			X			X			X			X				X
BRIEF [s]		X									X			X			X			X			X			X				X
Weekly call (non-visit weeks) [t]																														
Adverse events [u]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

Assessment [a]	Study Week	73[b]	74	75	76	77	78	79	80	81	85	89	93	97	101	105	109	113	117	121	125	129	133	137	141	145	149	153	HRV[c]	SC/ET[d]
	Study Day	505	512	519	526	533	540	547	554	561	589	617	645	673	701	729	757	785	813	841	869	897	925	953	981	1009	1037	1065		
	Window (Days)	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7		
Concomitant medication [u]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Administer study drug [v]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		

ACTH, adrenocorticotropic hormone; ADHD-RS IV, attention deficit hyperactivity disorder rating scale IV; BRIEF, Behavior Rating Inventory of Executive Function; ECG, electrocardiogram; eCRF, electronic Case Report Form; HRV, hypersensitivity reaction visit; NSAID, non-steroidal anti-inflammatory drug; PE, physical examination; PK, pharmacokinetics; PKU, phenylketonuria; SC/ET, Study Completion/Early Termination visit.

[a] Scheduled visits take place at the clinic or, with the approval of the investigator or designee, virtually. For virtual visits the investigator/designee performs assessments while online with the subject, apart from specimen collections and vital signs, which are performed by home healthcare personnel. Non-clinic visits are via telephone. Assessments are performed pre-dose unless otherwise specified.

[b] For Cohort B control subjects, the Week 73 visit marks both the end of Part 1, when their PKU is treated with diet alone, and the beginning of Part 2, when they receive pegvaliase. The Week 73 pre-dose assessments constituting the end of Part 1 are shown in the Week 73 column of Table 1.3.3. These same assessments, used as baseline assessments for Part 2, are shown in the Week 73 column of Table 1.3.4, along with the administration of study drug that marks the beginning of Part 2.

[c] If the investigator determines that the NCI-CTCAE Grade ≥ 3 hypersensitivity reaction is related to administration with study drug, the subject will be asked to return to the clinic within 24 hours of event onset for a hypersensitivity reaction visit (HRV). Sampling for anti-pegvaliase IgE should be performed 8 to 24 hours after event onset and before the next dose of study drug.

[d] Subjects should perform the Study Completion (SC) Visit 4 weeks after the last dose of study drug or after completion of study participation, whichever occurs last. For subjects who terminate from the study early, the Early Termination (ET) Visit occurs within 4 weeks of the last study drug dose or after withdrawal from study participation, whichever occurs last. Subjects who discontinue from study drug early will be asked to continue to complete the remaining study assessments until study completion as long as such continued participation will not detrimentally affect the health, safety, and welfare of the subject per investigator determination.

[e] Once subject/caregiver competence is documented, the subject/caregiver administers study drug daily.

[f] Must be completed prior to administration of the first dose of study drug. Additional observers identified after screening must be trained.

[g] A full PE will be performed at Week 73, Week 145, and the SC/ET visit. A brief PE will be performed at other visits. A full physical examination will include, at a minimum, general appearance (head, eyes, ears, nose, and throat), and an assessment of cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, musculoskeletal, and neurologic systems. A brief physical examination may be performed at the discretion of the investigator based on the subject's clinical condition and may include, but is not limited to, general appearance and an assessment of cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, musculoskeletal, and neurologic systems.

[h] It is recommended that urine samples are obtained as a first or second morning void. In the event of elevated urinary protein on a test result, a repeat urinalysis should be performed. This repeat urine sample must be performed in the morning at the first or second morning void to allow for accurate test results and may be performed by a home healthcare nurse.

[i] To be done by a local laboratory.

[j] Serum cortisol samples should be taken before study drug administration in the morning before 10:00 am. A home healthcare nurse may collect samples for subject convenience. If 2 results are low and abnormal, the subject will be asked to perform additional sampling for plasma ACTH and a low-dose conventional ACTH stimulation test or the ACTH test method based on the clinical practice at the site.

[k] It is recommended that urine samples be obtained as a first or second morning void. Subjects with a confirmed urine/albumin creatinine ratio of ≥ 100 mg/g should be referred to a nephrologist for consultation if results were within normal range at Baseline. Subjects who had elevated results at Baseline followed by a confirmed subsequent increase of 100-200 mg/g from Baseline should also be referred to a nephrologist for consultation.

[l] To be done locally (if applicable). If urine pregnancy test is positive or equivocal, serum pregnancy test (central laboratory) must be done.

[m] Subjects should record at home all food, beverages, special low-protein foods, and medical foods consumed during the 3 consecutive days preceding a study visit. No change in protein (dietary and medical food) intake is allowed in Part 1.

[n] Blood is collected before study drug administration (pre-dose). Intensive PK sampling will be performed at Week 145 and at dose escalation visits in all subjects. Samples will be taken at pre-dose, 2, 4, 8, 12, and 24 hours post dose. The 24-hour sample will be taken prior to the next daily dose.

[o] Sample collection should be performed where allowed by country-specific regulations and EC/IRB.

[p] Blood is collected for blood Phe analysis after fasting a minimum of 2.5 hours. In addition to the blood draws for blood Phe analysis collected in the clinic and shown in the SoA, there are 5 required blood collections that may be drawn either at the clinic or elsewhere (eg, at home). These are to occur at Weeks 83, 87, and 91, and 2 weeks prior to the planned escalations to 40 and 60 mg/day.

[q] Immunogenicity assays include total anti-pegvaliase antibodies (TAb), anti-PAL IgG, anti-PAL IgM, anti-PEG IgM, anti-PEG IgG, and neutralizing antibodies (NAb). At the HRV, only anti-pegvaliase IgE will be assessed.

[r] Completed by parent/guardian.

[s] Completed by parent/guardian.

[t] Between scheduled clinic visits, the clinic staff will contact the subject weekly to monitor if the subject is experiencing problems with drug administration, to ask about any AEs or concomitant medications, and to answer questions.

[u] AEs and concomitant medications should be noted whenever a subject is assessed by study personnel. Beginning with start of study treatment, All AEs and SAEs are collected until 4 weeks after the last study drug dose or the Study Completion Visit/Early Termination visit, whichever occurs last. If there is a severe (Grade 3) injection site reaction, the subject may be referred to a dermatologist for consultation and a skin biopsy (optional; if clinically indicated). If photographs of the severe injection site reaction are taken, they may be collected by the sponsor.

[v] Subjects must be premedicated with an H1 antagonist, and an H2 antagonist, and an antipyretic approximately 2 to 3 hours prior to each dose of study drug until they reach the Maintenance Phase, defined as when subjects achieve blood Phe ≤ 600 µmol/L while they are on a stable dose of pegvaliase. Premedication may be considered during the Maintenance Phase at the investigator's discretion, eg, approximately 2 to 3 hours prior to study drug for 1 week upon reintroduction of study drug, upon resolution of an AE, following any dose interruption of ≥ 4 days, and for any dose increases in the Extension Phase. Subjects may also be premedicated at any other time in the study per investigator discretion. When an NSAID is administered as a premedication, it should be given with food. For non-clinic visits, subjects are asked about study drug administration.

[j] Serum cortisol samples should be taken before study drug administration in the morning before 10:00 am. A home healthcare nurse may collect samples for subject convenience. If 2 results are low and abnormal, the subject will be asked to perform additional sampling for plasma ACTH and a low-dose conventional ACTH stimulation test or the ACTH test method based on the clinical practice at the site.

[k] It is recommended that urine samples be obtained as a first or second morning void. Subjects with a confirmed urine/albumin creatinine ratio of ≥ 100 mg/g should be referred to a nephrologist for consultation if results were within normal range at Baseline. Subjects who had elevated results at Baseline followed by a confirmed subsequent increase of 100-200 mg/g from Baseline should also be referred to a nephrologist for consultation.

[l] To be done locally (if applicable). If urine pregnancy test is positive or equivocal, serum pregnancy test (central laboratory) must be done.

[m] Subjects should record at home all food, beverages, special low-protein foods, and medical foods consumed during the 3 consecutive days preceding a study visit. No change in protein (dietary and medical food) intake is allowed in Part 1.

[n] Blood is collected before study drug administration (pre-dose). Intensive PK sampling will be performed at Week 145 and at dose escalation visits in all subjects. Samples will be taken at pre-dose, 2, 4, 8, 12, and 24 hours post dose. The 24-hour sample will be taken prior to the next daily dose.

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[p] Blood is collected for blood Phe analysis after fasting a minimum of 2.5 hours. In addition to the blood draws for blood Phe analysis collected in the clinic and shown in the SoA, there are 5 required blood collections that may be drawn either at the clinic or elsewhere (eg, at home). These are to occur at Weeks 83, 87, and 91, and 2 weeks prior to the planned escalations to 40 and 60 mg/day.

[q] Immunogenicity assays include total anti-pegvaliase antibodies (TAb), anti-PAL IgG, anti-PAL IgM, anti-PEG IgM, anti-PEG IgG, and neutralizing antibodies (NAb). At the HRV, only anti-pegvaliase IgE will be assessed.

[r] Completed by parent/guardian.

[s] Completed by parent/guardian.

[t] Between scheduled clinic visits, the clinic staff will contact the subject weekly to monitor if the subject is experiencing problems with drug administration, to ask about any AEs or concomitant medications, and to answer questions.

[u] AEs and concomitant medications should be noted whenever a subject is assessed by study personnel. Beginning with start of study treatment, All AEs and SAEs are collected until 4 weeks after the last study drug dose or the Study Completion Visit/Early Termination visit, whichever occurs last. If there is a severe (Grade 3) injection site reaction, the subject may be referred to a dermatologist for consultation and a skin biopsy (optional; if clinically indicated). If photographs of the severe injection site reaction are taken, they may be collected by the sponsor.

[v] Subjects must be premedicated with an H1 antagonist, and an H2 antagonist, and an antipyretic approximately 2 to 3 hours prior to each dose of study drug until they reach the Maintenance Phase, defined as when subjects achieve blood Phe ≤ 600 µmol/L while they are on a stable dose of pegvaliase. Premedication may be considered during the Maintenance Phase at the investigator's discretion, eg, approximately 2 to 3 hours prior to study drug for 1 week upon reintroduction of study drug, upon resolution of an AE, following any dose interruption of ≥ 4 days, and for any dose increases in the Extension Phase. Subjects may also be premedicated at any other time in the study per investigator discretion. When an NSAID is administered as a premedication, it should be given with food. For non-clinic visits, subjects are asked about study drug administration.

2 INTRODUCTION

Phenylketonuria (PKU, OMIM 261600) is a rare autosomal recessive genetic disorder that is characterized by an inability of the body to break down the amino acid, phenylalanine (Phe), and is caused by mutations in the gene encoding phenylalanine hydroxylase (PAH). PAH catalyzes the conversion of the essential amino acid Phe to tyrosine, and this enzymatic activity is facilitated by tetrahydrobiopterin (BH4). PAH deficiency results in abnormally elevated concentrations of Phe. The estimated incidence of PKU is 1 in 15,000 births in the United States (US) (NIHCDP 2001).

The pathophysiology of PKU is well understood. A substantial body of evidence confirms that elevated Phe is directly toxic to cells in the brain, where it causes inhibition of protein synthesis in humans and animals (Wall 1990), affects the normal morphology of myelinating proteins (Dyer 1996), and leads to arrested or delayed development of dendrites and synapses in the cerebral cortex (Huttenlocher 2000). Neuropathology in PKU has been shown to derive from oxidative stress, impaired neurotransmitter metabolism, impaired protein synthesis and lipid metabolism, and brain energy metabolism alterations (Schuck 2015). The neurotoxicity of elevated Phe is a direct effect of Phe itself (Kaufman 1989). No abnormalities in Phe metabolites are observed in patients with PKU and normal Phe metabolites are toxic only at concentrations that are much higher than those reported in individuals with PKU.

With newborn screening followed by Medical Nutritional Therapy (MNT), the most severe manifestation of the disease, mental retardation, is prevented. Phe restriction with MNT involves consumption of semisynthetic medical foods that provide a source of Phe-free protein, modified low-protein foods, a small amount of natural protein (to provide required amounts of Phe), and other supplements (eg, vitamins and minerals) to correct nutritional deficiencies that can result from Phe restriction. Without highly restricted Phe intake and MNT, individuals with PKU quickly reach and sustain toxic concentrations of blood Phe. Individuals with classic PKU may have a complete absence or profound deficiency of PAH and typically show very high elevations of blood Phe (≥ 1200 µmol/L).

Historically, it was recommended that adherence to the Phe-restricted diet could end after age 6, but later studies have shown that patients who maintained Phe control longer had better outcomes in neuropsychological performance and areas of attention and speed of processing (Cerone 1999; Channon 2007; Rey 1996). A robust body of research suggests that early blood Phe control through dietary management is imperative in PKU populations, and that adult intelligence quotient (IQ) levels are correlated with the duration and vigilance of dietary management in childhood and adolescence (Pietz 1997; Smith 2000; Stemerdink 2000; Waisbren 1999).

To be optimally effective, a PKU diet needs to be started immediately after birth and continued for life. The American College of Medical Genetics and Genomics (ACMG) practice guidelines for PKU recommend life-long management and maintenance of metabolic control as essential to optimal functioning of individuals with PKU, with a goal of maintaining blood Phe concentrations ≤ 360 µmol/L (Vockley 2014). Similar European guidelines for the management of PKU recommend treatment target Phe levels of ≤ 600 µmol/L for patients older than 12 years (van Spronsen 2017).

Compliance with diet is a significant component of disease management, but life-long dietary compliance can be extremely difficult. A study conducted over 6 years at 4 treatment centers showed that dietary compliance in PKU populations was found to decline with age. Specifically, during infancy and in pre-school aged children, compliance with restrictive PKU diet is high because their diet is carefully managed by a parent and nutritionist. However, when PKU children start school, their blood Phe levels increase because of changes in socialization and changes in their palate, making compliance to a Phe-restricted diet more challenging (Walter 2002; Walter 2004). As children age, they become more likely to find formula and synthetic low-Phe foods distasteful and they become exposed to a broader range of food and peer pressure, both of which encourage noncompliance (MacDonald 2010). Adolescents/young adults with PKU are at particular risk for non-compliance with MNT as they transition away from childhood when parental oversight of dietary control decreases (Brown 2016). In addition, adolescents/young adults who have stopped MNT and attempt to resume a Phe-restricted diet into adulthood have difficulty maintaining the diet, with 31% of patients no longer maintaining dietary control after 10 months of restarting (Schuett 1985).

Therefore, despite the best efforts of the medical community and patients, life-long therapy with MNT with Phe restriction is not sustainable nor feasible as a treatment option for the majority of patients with PKU (Walter 2002; Burton 2010; Aoki 2007). The inability to adhere to MNT results in uncontrolled blood Phe levels associated with executive dysfunction, depression, and a variety of behavioral and psychiatric problems (Moyle 2007; Pietz 1997; Smith 2000; Waisbren 1999). Adolescents with PKU with poor metabolic control represent a patient population in great need of additional therapeutic options.

BioMarin has developed pegvaliase as a subcutaneous enzyme substitution treatment to reduce blood Phe levels in adult patients with PKU. Enzyme replacement therapy with PAH is not feasible due to the instability of PAH in plasma and the difficulty with efficiently delivering an intact, active form of the enzyme to the liver (Harding 2003; Kim 2004). Pegvaliase is a genetically modified phenylalanine ammonia lyase (PAL) enzyme product of

the cyanobacterium *Anabaena variabilis*, which is PEGylated to decrease immunogenicity and increase half-life. Unlike PAH, the PAL enzyme is active in plasma and, independently of cofactor BH4 which is required for PAH activity, catalyzes Phe to trans-cinnamic acid (t-CA) and ammonia, which are metabolized in the liver and excreted in the urine, respectively. The pegvaliase mechanism of action corrects the underlying PAH deficiency that leads to inability to metabolize Phe, increased Phe levels in the body, and consequent neurocognitive, neuropsychiatric, and executive functioning deficits.

A comprehensive review of pegvaliase is contained in the Investigator's Brochure (IB). Investigators must review this document prior to initiating this study.

2.1 Study Rationale

Although it is recommended that adolescents with PKU maintain good metabolic control as they move into adulthood (Vockley 2014), in separate surveys > 60% of adolescents (13 to 17 years of age) in the US have blood Phe concentrations above recommended targets (Jurecki 2017) and 79% of patients 15 to 19 years report blood Phe concentrations above targets (Walter 2002). Adolescents aged 12 to 17 years with PKU report difficulties with maintaining blood Phe control as they transition away from childhood when parental oversight of dietary and blood Phe control decreases (Brown 2016; van Spronsen 2017). In a 2015 National PKU Alliance (NPKUA) survey conducted among over 625 adults and families, approximately 25% of those caregivers indicated that their children's average blood Phe levels were in excess of the ACMG-recommended targeted range (120 to 360 µmol/L), and over half of all respondents (adults and caregivers) reported that it was somewhat to very difficult to manage their PKU (Brown 2016).

Adolescent patients report difficulties adhering to a Phe-restricted diet to maintain blood Phe levels within a safe range (Brown 2016; van Spronsen 2017), and a clinic survey assessing compliance with clinical guidance and dietary restriction showed that 60% of adolescents aged 13 to 17 were non-compliant, with an associated increase of Phe levels to > 360 µmol/L in 50% of patients and Phe levels > 600 µmol/L in 22% of patients. The same survey demonstrated that non-compliance starts as early as ages 5 to 12, and non-adherence to guidelines increases with age.

For adolescent patients at increased risk for loss of metabolic control maintained during childhood, a variety of adverse neuropsychological deficits (including deficits in executive functioning and psychiatric symptoms, such as anxiety, depression, and phobias) develop later in life when there is relaxation of blood Phe control (Huijbregts 2018; Diamond 1997). Adolescent PKU patients with blood Phe levels ≤360 µmol/L have been shown to perform better at neuropsychological and neurocognitive tasks (Huijbregts 2002; Jahja 2014).

The approval of Palynziq addressed a previous unmet medical need, providing adult PKU patients with a treatment that substantially reduced blood Phe concentrations and resulted in the stabilization of Phe above and beyond diet. The reduction and stabilization of blood Phe were associated with improvement in unmanaged symptoms, including neurocognitive symptoms. There is potential for improvement in these outcomes for adolescents as well given the demonstrated efficacy in adults. Therefore, expansion of Palynziq use to include adolescent patients 12 to 17 years with uncontrolled blood Phe concentrations greater than 600 µmol/L on existing management would broaden the patient population who can benefit from Palynziq treatment and address a currently underserved patient population.

The pathogenesis and pathophysiology of PKU, driven by an absence or deficiency in PAH enzyme leading to high Phe concentration, is applicable to both adolescents and adults. The pegvaliase mechanism of action to correct the underlying metabolic deficiency of PAH gene is the same in both adults and adolescents. Adolescent PKU patients are not expected to have specific ongoing developmental changes that could result in a different safety or efficacy outcome with pegvaliase treatment.

2.2 Background

Palynziq received marketing authorization in the US and European Union (EU). The original Biologics License Application (BLA) for Palynziq was approved by the Food and Drug Administration (FDA) at dosages up to 40 mg/day on 24 May 2018, to reduce blood Phe concentrations in adult patients with phenylketonuria (PKU) who have uncontrolled blood Phe concentrations greater than 600 µmol/L on existing management (BLA STN 761079). A supplemental Biologics License Application (sBLA) that included the addition of the maximum maintenance dose of 60 mg/day and long-term safety and efficacy data for adults was approved by the FDA on 06 October 2020. A Risk Evaluation and Mitigation Strategy (REMS) is a required element of the Palynziq approval in the US. Palynziq was approved by the European Commission on 03 May 2019 at dosages up to 60 mg/day to reduce blood Phe concentrations in patients aged 16 years and older who have inadequate blood Phe control (defined as blood Phe levels greater than 600 µmol/L) despite prior management with available treatment options.

BioMarin recognizes that PKU is a serious genetic disease diagnosed at birth that requires strict management of blood Phe levels from birth and continuing through life. Now that a positive benefit to risk profile has been established in adults, BioMarin is proposing this pegvaliase clinical study in adolescent PKU patients 12 to 17 years of age.

2.3 Benefit/Risk Assessment

Regulatory approval of Palynziq in the US and the EU was based on demonstration of efficacy from the Phase 2 and Phase 3 data of the pegvaliase clinical development program. The approval of pegvaliase addressed a previous unmet medical need, providing adult PKU patients with a treatment that substantially reduced blood Phe concentrations and resulted in the stabilization of Phe above and beyond diet. This reduction and stabilization of blood Phe was also associated with improvement in unmanaged symptoms including neurocognitive symptoms. Given the demonstrated efficacy in adults, there is the potential for improvement in these outcomes for adolescents.

The important identified risks for pegvaliase include acute systemic hypersensitivity reaction (equivalent to anaphylaxis for the purpose of this protocol; see Section 8.3.8.1 for how this risk is defined), angioedema, serum sickness, hypophenylalaninemia, persistent arthralgia, and severe injection site reaction (see Section 8.3.8 for event definitions). These events have been effectively treated and managed in the clinical trial and post-marketing settings. Healthcare providers, patients, and observers are trained in the recognition and management of both an acute systemic hypersensitivity reaction or angioedema, including the use of injectable epinephrine (adrenaline) and the need to seek immediate medical assistance. Premedication is required during Induction/Titration Dosing to help mitigate the risk of hypersensitivity reactions, including acute systemic hypersensitivity reactions. While hypophenylalaninemia has not been reported frequently in the post-marketing setting, it was observed during the clinical studies, where it was managed with decreases in pegvaliase dosing and increases in dietary protein intake. Subjects will undergo frequent blood Phe monitoring during induction and titration and throughout dosing and will be required to dose reduce with confirmed hypophenylalaninemia (ie, 2 consecutive blood Phe levels < 30 µmol/L).

The important potential risks of treatment with pegvaliase also include potential complications of immune complex formation resulting in end-organ damage, fetal developmental toxicity, and acute systemic hypersensitivity reaction in patients taking other concurrent injectables containing PEG. No evidence of immune complex-mediated end organ damage or any early clinical signs potentially related to immune complex mediated disease have been reported in the clinical trial or commercial setting. Concurrent injectables containing PEG are prohibited during study treatment with the exception of COVID vaccinations (see Section 6.8.1).

The potential risks are justified by the anticipated benefits that may be afforded to adolescent subjects. Each subject will have a comprehensive plan that includes frequent monitoring of

adverse events, blood Phe levels, and pregnancy during the study. Safety will be assessed by adverse event reporting and clinical laboratory assessments.

More detailed information about the known and expected benefits and risks and reasonably expected adverse events of pegvaliase may be found in the IB.

2.3.1 Risk Assessment

Table 2.3.1.1: Risks Associated with Study Drug

Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Acute systemic hypersensitivity reaction	In the completed Phase 2 and Phase 3 clinical trials, 16 out of 285 (6%) subjects experienced 25 episodes of acute systemic hypersensitivity reactions of any severity. Acute systemic hypersensitivity reactions were managed by administration of adrenaline (44%; 11/25 episodes), corticosteroids (56%; 14/25 episodes), antihistamines (56%; 14/25 episodes), and/or oxygen (8%; 2/25 episodes). Four out of 16 (1%; 4/285) subjects experienced a total of 5 episodes of an acute systemic hypersensitivity reaction that were considered severe. The underlying mechanism for acute systemic hypersensitivity reaction observed in clinical trials was non-IgE mediated Type III (immune-complex mediated) hypersensitivity. Acute systemic hypersensitivity reaction was most frequent during the Induction/Titration Phase (5% of subjects; 19 episodes over mean treatment duration of 13 months) and decreased in the Maintenance Phase (2% of subjects; 6 episodes over a mean treatment duration of 33 months). The risk of an acute systemic hypersensitivity reaction occurring is 6-fold higher in the Induction/Titration Phase compared to the Maintenance Phase.	To mitigate the risk of an acute systemic hypersensitivity reaction, an adult observer is required for a minimum of 1 hour post injection and premedication including H1 and H2 antagonists and an antipyretic is required 2 to 3 hours before each injection until subjects reach the Maintenance Phase.[a] Premedication may be considered during the Maintenance Phase at the investigator's discretion. Subjects will be provided with 2 epinephrine injectors and instructed to carry both injectors with them at all times. Subjects and adult observers will be trained to recognize the signs and symptoms of anaphylaxis and, if these reactions occur, to call for emergency medical support and administer an epinephrine injector. For the duration of the study, each subject will be contacted weekly by study site personnel to monitor for drug administration problems and adverse events (AEs). Risk mitigation measures also include dosing instructions for other hypersensitivity adverse events (Table 6.5.1.1).
Angioedema	In the completed Phase 2 and Phase 3 clinical trials, 21 out of 285 (7%) subjects experienced 37 episodes of mild to moderate episodes of angioedema (symptoms include one or more of the following: pharyngeal edema, swollen tongue, lip swelling, mouth swelling, eyelid edema and face edema) occurring independent of acute systemic	To mitigate the risk of hypersensitivity reactions, premedication including H1 and H2 antagonists and an antipyretic is required 2 to 3 hours before each injection until subjects reach the Maintenance Phase.[a] Premedication may be considered during the maintenance phase at the investigator's discretion.

Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
	hypersensitivity reaction. Angioedema was most frequent during the Induction/Titration Phase (6% of subjects; 27 episodes over mean treatment duration of 13 months) and decreased in the Maintenance Phase (3% of subjects; 10 episodes over mean treatment duration of 33 months). The risk of angioedema occurring is 4.5-fold higher in the Induction/Titration Phase compared to the Maintenance Phase.	An adult observer is required for a minimum of 1 hour post injection throughout the study. For the duration of the study, each subject will be contacted weekly by study site personnel to monitor for drug administration problems and AEs. Risk mitigation measures also include dosing instructions for hypersensitivity adverse events (Table 6.5.1.1).
Serum sickness	In the completed Phase 2 and Phase 3 clinical trials, serum sickness was reported in 7 out of 285 (2%) subjects. Serum sickness was most frequent during the Induction/Titration Phase (2% of subjects; 6 episodes over mean treatment duration of 13 months) and decreased in the Maintenance Phase (0.6% of subjects; 1 episode over mean treatment duration of 33 months). The risk of serum sickness occurring is greater than 2-fold higher in the Induction/Titration Phase compared to the Maintenance Phase.	To mitigate the risk of hypersensitivity reactions, premedication including H1 and H2 antagonists and an antipyretic is required 2 to 3 hours before each injection until subjects reach the Maintenance Phase of dosing.[a] Premedication may be considered during the Maintenance Phase at the investigator's discretion. An adult observer is required for a minimum of 1 hour post injection throughout the study. For the duration of the study, each subject will be contacted weekly by study site personnel to monitor for drug administration problems and AEs. Risk mitigation measures also include dosing instructions for hypersensitivity adverse events (Table 6.5.1.1).
Persistent arthralgia (> 6 month duration)	In the completed Phase 2 and Phase 3 clinical trials, 245 out of 285 (86%) subjects experienced episodes consistent with arthralgia (including back pain, musculoskeletal pain, pain in extremity, and neck pain). Arthralgia occurred as early as the first dose and can occur at any time during treatment. Arthralgia was most frequent during the Induction/Titration Phase (79% of subjects; 1269 episodes over mean treatment duration of 13 months) and decreased in the Maintenance Phase (67% of subjects; 673 episodes over mean treatment duration of 33 months). The risk of arthralgia occurring is 3.1-fold higher in the Induction/Titration Phase compared to the Maintenance Phase. Seven (7%) percent of the subjects experienced persistent arthralgia (≥ 6 months). The risk of persistent	To mitigate the risk of hypersensitivity reactions, premedication including H1 and H2 antagonists and an antipyretic is required 2 to 3 hours before each injection until subjects reach the Maintenance Phase of dosing.[a] Premedication may be considered during the Maintenance Phase at the investigator's discretion. An adult observer is required for a minimum of 1 hour post injection throughout the study. For the duration of the study, each subject will be contacted weekly by study site personnel to monitor for drug administration problems and AEs. Risk mitigation measures also include dosing instructions for hypersensitivity adverse events (Table 6.5.1.1).

Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
	arthralgia may have significant impact on quality of life. Severe arthralgia (severe pain limiting self-care activities of daily living) was experienced in 5% of subjects. Dose was not changed for 96% of episodes and all persistent arthralgia episodes resolved without sequelae.	
Severe injection site reaction	Injection site reaction (ISR) was reported in 266 out of 285 (93%) subjects. The most common ISR (occurring in at least 10% of subjects) was reaction, erythema, bruising, pruritus, pain, swelling, rash, induration, and urticaria. Injection site reaction was most frequent during the Induction/Titration Phase (90% of subjects; 3908 episodes over mean treatment duration of 13 months) and decreased in the Maintenance Phase (66% of subjects; 1226 episodes over mean treatment duration of 33 months). The risk of an ISR occurring is 5.2-fold higher in the Induction/Titration Phase compared to the Maintenance Phase. Three ISRs consistent with granulomatous skin lesions were reported (each reaction occurring in 1 patient): granulomatous dermatitis (occurred 15 months after pegvaliase treatment and lasted 16 days), xanthogranuloma (occurred 12 months after pegvaliase treatment and lasted 21 months), and necrobiosis lipoidica diabeticorum (occurred 9 months after pegvaliase treatment and lasted 9 months). Necrobiosis lipoidica diabeticorum was treated with steroid injections and was complicated by a *Pseudomonas* infection. All of these injection site reactions resolved.	To mitigate the risk of hypersensitivity reactions, premedication including H1 and H2 antagonists and an antipyretic is required 2 to 3 hours before each injection until subjects reach the Maintenance Phase of dosing.[a] Premedication may be considered during the Maintenance Phase at the investigator's discretion. An adult observer is required for a minimum of 1 hour post injection throughout the study. For the duration of the study, each subject will be contacted weekly by study site personnel to monitor for drug administration problems and AEs. Risk mitigation measures also include dosing instructions for hypersensitivity adverse events (Table 6.5.1.1). Any subject who experiences a severe (Grade 3) skin reaction will be asked to consult with a dermatologist for diagnosis and, if clinically indicated, a skin biopsy will be recommended. A Skin Reaction eCRF must be completed.
Hypophenylalaninemia	In the completed Phase 2 and Phase 3 clinical trials, 46% of the subjects developed hypophenylalaninemia (blood phenylalanine levels below 30 µmol/L on two consecutive measurements). The risk of hypophenylalaninemia occurring is 2.1-fold higher in the maintenance phase compared to the Induction/Titration phase. In the completed adult clinical studies, dose reductions were effective in	Blood Phe levels will be frequently monitored during induction and titration dosing and regularly monitored during maintenance dosing in both the Primary Treatment Phase and the Extension Phase of the study. Risk mitigation measures, including protocol-required adjustments in the pegvaliase dose and/or dietary intake of Phe, will be implemented to mitigate the potential risk of chronic

Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
	managing hypophenylalaninemia in subjects experiencing hypophenylalaninemia despite appropriate levels of protein intake.	hypophenylalaninemia levels (< 30 µmol/L) in adolescent subjects.
Complications of immune complex formation resulting in end-organ damage	No evidence from clinical trials or post-marketing reports of immune complex-mediated end organ damage or any early clinical signs potentially related to immune complex mediated disease.	Risk mitigation measures include monitoring clinical laboratory tests (chemistry, hematology, urinalysis) and adverse events for immune complex-mediated end-organ damage. These mitigations will also allow early detection of adverse effects of PEG accumulation. For the duration of the study, each subject will be contacted weekly by study site personnel to monitor for AEs.
Fetal development toxicity	There are limited data from the use of pegvaliase in pregnant women. Animal studies have shown maternal reproductive toxicity that was associated with decreased blood phenylalanine concentrations below normal levels. Uncontrolled blood phenylalanine levels (hyperphenylalaninemia) before and during pregnancy are associated with increased risk for miscarriage, major birth defects (including microcephaly and major cardiac malformations), intrauterine fetal growth retardation and future intellectual disability with low IQ. Additional risk to the unborn child due to hypophenylalaninemia is not established.	If sexually active, male or female subjects must not plan to become pregnant (self or partner) and must use 2 acceptable methods of contraception while participating in the study beginning at Screening and for 4 weeks after discontinuing study drug. Contraceptive use must be consistent with the guidance provided in Appendix 4, Section 10.4 of this protocol.
Unpredictable immune-mediated response in patients < 16 years	The adult development program was restricted to subjects aged > 16 years of age. Review of spontaneous adverse reaction reports in patients < 16 years of age, using Palynziq in the commercial setting has not identified any unpredictable immune responses. The adverse reactions reported in patients < 16 years of age are consistent with the known safety profile of Palynziq as presented in the USPI and SmPC.	To mitigate the risk of hypersensitivity reactions, premedication including H1 and H2 antagonists and an antipyretic is required 2 to 3 hours before each injection until subjects reach the Maintenance Phase. [a] Premedication may be considered during the Maintenance Phase at the investigator's discretion. An adult observer is required for a minimum of 1 hour post injection throughout the study. For the duration of the study, each subject will be contacted weekly by study site personnel to monitor for drug administration problems and AEs. Risk mitigation measures also include dosing instructions for hypersensitivity adverse events (Table 6.5.1.1).

Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Acute systemic hypersensitivity reactions in patients taking other concurrent injectables containing PEG	PEGylated proteins have the potential to elicit an immune response. Because antibodies bind to the PEG portion of pegvaliase, there may be potential for binding with other PEGylated therapeutics and increased hypersensitivity to other PEGylated injectables. In a single dose study of pegvaliase in adult subjects with PKU, two patients receiving concomitant injections of medroxyprogesterone acetate suspension containing PEG experienced hypersensitivity reactions.	Risk mitigation measures include prohibiting any injectable drugs containing polyethylene glycol (PEG; other than pegvaliase), including medroxyprogesterone injection, within 3 months prior to the start of Screening/Run-in and during study participation with the exception of COVID vaccinations (see Section 6.8.1).

[a] Subjects are in the Maintenance Phase when they achieve blood Phe ≤600 µmol/L while they are on a stable dose of pegvaliase. This is distinct from Maintenance Dosing, defined as the period following Induction/Titration Dosing when Maintenance Doses (20, 40, or 60 mg/day) are administered, as outlined in Table 6.5.1.

2.3.2 Benefit Assessment

The overall clinical benefit of pegvaliase was established in patients with PKU based on data from completed Phase 2 and Phase 3 clinical studies. The approval of Palynziq addressed a previous unmet medical need, providing adult PKU patients with a treatment that substantially reduced blood Phe concentrations and resulted in the stabilization of Phe above and beyond diet. This reduction and stabilization was also associated with improvement in unmanaged symptoms including neurocognitive symptoms. Given the demonstrated efficacy in adults, there is the potential for improvement in these outcomes for adolescents.

2.3.3 Overall Benefit/Risk Conclusion

Results from the robust pegvaliase clinical development program demonstrated that pegvaliase treatment up to 60 mg/day demonstrated a positive benefit-risk profile in patients with PKU with uncontrolled blood Phe levels > 600 µmol/L.

Taking into account the measures taken to minimize risk to subjects participating in this study, the anticipated benefits that may be afforded to adolescent PKU patients treated with pegvaliase outweigh the potential risks.

3 OBJECTIVES AND ENDPOINTS

The primary, secondary, and exploratory objectives and associated endpoints are shown in Table 3.1.

Table 3.1: Objectives and Endpoints

Objectives	Endpoints
Primary	
To evaluate the efficacy of pegvaliase in adolescent subjects with PKU	The primary efficacy endpoint is change in blood Phe concentration from treatment naïve baseline following 72 weeks on study. Change in blood Phe from baseline is compared between subjects in the active (pegvaliase) and the control (diet-only) treatment arms (see Section 9.4.2).
To evaluate the safety of pegvaliase in adolescent subjects with PKU	• Adverse events (AEs), including serious AEs (SAEs) • Clinical laboratory tests (chemistry, hematology, and urinalysis) results • Vital signs • Physical examination (including height, weight) • Electrocardiogram (ECG) test results • Immunogenicity test results (anti-PEG IgG, anti-PEG IgM, anti-PAL IgG, anti-PAL IgM, TAb, NAb, and anti-pegvaliase IgE [hypersensitivity reaction visits only]).
Secondary	
To characterize total dietary protein intake in adolescent subjects with PKU after pegvaliase treatment	Total dietary protein intake must remain stable for change in blood Phe, the primary endpoint, to be accurately assessed. To gauge the stability of total protein intake, the change from baseline at Week 73 in total dietary protein intake (defined as the sum of the protein intake from medical food and intact food) will be measured (see Section 9.4.3).
To characterize the pharmacokinetics (PK) of pegvaliase in adolescent subjects with PKU	Trough plasma PK samples will be collected and analyzed as specified in the SoA. PK parameters assessed with intensive PK sampling will include $AUC_{0\text{-}24hr,ss}$, $C_{max,ss}$, $T_{max,ss}$, $t_{1/2}$, $CL_{ss/F}$, and $V_{dz/F}$. For subjects randomized to the active (pegvaliase) treatment arm, intensive PK sampling will be performed at Week 73 and for dose escalations to 20, 40, and 60 mg/day. For subjects randomized to the control (diet-only) treatment arm, there are no PK assessments in Part 1. In Part 2, intensive PK sampling will be performed at Week 145 and for dose escalations to 20, 40, and 60 mg/day (see Section 8.4).

Objectives	Endpoints
Exploratory Objectives	
To evaluate the effect of pegvaliase treatment on neurocognitive outcomes in adolescent subjects with PKU.	• The ADHD Rating Scale IV inattention subscale (ADHD-RS IV) rated by the parent/guardian and performed at Baseline (Day 1, pre-dose) and every 12 weeks throughout the study (see Section 8.1.3.1.1). • The Behavior Rating Inventory of Executive Function (BRIEF) rated by the parent/guardian and performed at Baseline (Day 1, pre-dose) and every 12 weeks throughout the study (see Section 8.1.3.1.2).
Explore the biochemical, molecular, and cellular aspects of PKU.	Blood will be collected to evaluate biochemical, molecular, and cellular aspects of PKU and to develop the assays used for these evaluations.
PAH genotyping	Testing will be performed on samples collected Day 1 pre-dose where allowed by local health authorities and the EC/IRB.

4 STUDY DESIGN

4.1 Overall Design

This Phase 3 multi-center study is designed to evaluate the safety and efficacy of pegvaliase administered daily to adolescent subjects (ages 12-17) with PKU. Pegvaliase will be subcutaneously injected in the clinic or at home by a trained subject, caregiver, adult observer, or visiting healthcare provider. The study design is summarized in Figure 1.2.1.

The study will enroll approximately 54 US and EU subjects 12 to 17 years old, inclusive, who will be evaluated using an open-label, 2-arm, randomized control design, with diet-only management of PKU as the control. Subjects will be randomized in a 2:1 ratio to the active and control arms, respectively, with 36 subjects receiving pegvaliase and 18 subjects managing their PKU with diet alone.

The primary efficacy endpoint is the change from baseline in blood Phe following 72 weeks on study (the end of Part 1). Beginning Week 73, subjects in the active treatment arm will continue to receive pegvaliase in the Extension Phase (Part 2) for up to an additional 80 weeks (through Week 153) in order to evaluate the long-term safety and efficacy of pegvaliase in adolescent subjects with PKU. Subjects in the diet-only control arm will initiate pegvaliase treatment beginning Week 73 and, from Weeks 73 through 145, follow the same dosing and assessment schedule that the active subjects followed from Weeks 1 through 73, after which they will receive pegvaliase for up to 8 additional weeks (through Week 153).

Eligible subjects are required to have 2 blood Phe concentration measurements > 600 µmol/L during the Screening/Run-in Period (7 to 10 days apart) and an average blood Phe concentration > 600 µmol/L over the past 12 months prior to Screening based on available historical data. Once enrolled, patients will be dosed with pegvaliase according to the I/T/M dosing regimen based on the US package insert (USPI), with the guidance to increase dose depending on individual blood Phe lowering efficacy (per investigator's discretion). Dose should be increased to 40 mg/day if blood Phe is > 360 µmol/L after 24 weeks dosing at 20 mg/day, and increased to 60 mg/day if blood Phe is > 360 µmol/L after 16 weeks dosing at 40 mg/day. BioMarin recommends that the target blood Phe levels should be based on individual blood Phe lowering efficacy.

In Part 1 (through the Week 73 visit), randomized subjects in both the control (diet-only) and the active (pegvaliase) treatment arms will be required to maintain the same level of dietary protein intake they established during Screening/Run-in. In Part 2, subjects in the control (diet-only) arm must continue to maintain this prescribed level of protein intake, whereas subjects randomized to the active (pegvaliase) arm whose blood Phe concentration is

≤ 360 µmol/L for a minimum of 4 weeks may adjust dietary protein intake based on individual subject response to pegvaliase and guidance from the investigator.

4.2 Scientific Rationale for Study Design

This Phase 3 multi-center study is designed to evaluate the safety and efficacy of pegvaliase administered daily to adolescent subjects (ages 12-17) with PKU. Approximately 54 US and EU subjects 12 to 17 years old, inclusive, will be evaluated using an open-label, 2-arm, randomized control design, with diet-only management of PKU as the control.

A RCT design, with diet-only management of PKU as the control, will allow for robust data collection and minimize the risk of confounding factors influencing the results. An open-label diet-only control arm is the best comparator for this age group as daily placebo injections would place undue burden on adolescent PKU patients.

Except for subjects randomized to pegvaliase in Part 2 whose blood Phe concentration is ≤ 360 µmol/L for a minimum of 4 weeks, subjects in both the control (diet-only) and active (pegvaliase) treatment arms are required to maintain a consistent level of protein intake throughout the study (see Section 4.1 and Section 8.1.2). Subjects are required to maintain a consistent level of protein intake to ensure that the safety and efficacy endpoints are attributable to pegvaliase rather than to changes in dietary protein intake.

Pegvaliase will be subcutaneously injected in the clinic for the first 8 weeks and afterwards at the clinic or at home by a trained subject, caregiver, adult observer, or visiting healthcare provider. Administration at home makes daily treatment less burdensome for subjects, thereby improving treatment compliance.

An adult observer is required for a minimum of 1 hour post injection throughout the study. An adult observer is defined as someone who will be present with the subject during and after pegvaliase administration, is able to recognize the signs and symptoms of an anaphylaxis episode, and is able to call for emergency medical support and administer epinephrine, if warranted. The requirement for an adult observer was added to ensure that subjects self-administering pegvaliase would have help in recognizing and quickly responding to signs and symptoms of anaphylaxis should they emerge.

The primary efficacy endpoint is the change from baseline in blood Phe following 72 weeks on study (the end of Part 1). Blood Phe level is the established clinical marker used to diagnose PKU and assess disease control, and reduction of blood Phe concentration to target levels is the universally acknowledged treatment goal for PKU patients. Published literature support the clinical benefit of lowering blood Phe concentration to ≤ 600 µmol/L for associated improvement in clinical outcomes including cognitive performance, behavior and

mood (Palermo 2017; Romani 2017; Burgard 1999; Azen 1996; Smith 1989; Fitzgerald 2000; Costello 1994; Fonnesbeck 2013; Gassio 2003).

In addition, evidence has shown improved clinical benefit with even more aggressive blood Phe lowering targets. In children and adolescents with PKU, studies show that patients with blood Phe levels < 360 µmol/L perform better at neuropsychological and neurocognitive tasks (Huijbregts 2002; Jahja 2014). The findings from these studies demonstrate the need to adequately control blood Phe levels in PKU pediatric patients to below the 600 µmol/L threshold.

4.2.1 Subject Input into Design

BioMarin sought input from the PKU community in designing this study. Parents and a scientist from the National PKU Alliance reviewed portions of the protocol and provided feedback. Among other important input, the community stressed the importance of:

- Setting expectations for study participation, something that is critical to parents during the informed consent process
- Ongoing support from site personnel during the study
- Training for adult observers
- Simplifying, as much as possible, the diet tracking requirements given the time commitment required on the part of families
- Flexibility in scheduling clinic visits and including home and virtual visits where possible

BioMarin has taken this PKU community feedback into account when designing the study:

- Detailed training will be provided for subjects, parents, and adult observers, and will continue throughout the study.
- Diet diaries will be in paper format and, consistent with past studies, diet tracking is restricted to the 3 days prior to study visits.
- Scheduled visits will take place at the clinic or, with the approval of the investigator or designee, virtually. For virtual visits the investigator/designee performs assessments while online with the subject, apart from specimen collections and vital signs, which are performed by home healthcare personnel.

4.3 Justification for Dose and Dose Regimen

Pegvaliase is a daily treatment for patients with PKU that will be administered in an Induction/Titration/Maintenance (I/T/M) regimen described in Section 6.5. Previous clinical studies have suggested the primary clearance mechanism of pegvaliase from plasma is via formation of circulating immune complexes leading to complement activation and removal

of the drug by phagocytosis, indicating pegvaliase plasma exposure was driven by immune response. Considering that the immune system matures prior to adolescence (Olin 2018; Georgountzou 2017), the immune response to pegvaliase among adolescent subjects is expected to be similar to the responses observed in 16 to 57-year-old study subjects in the pegvaliase clinical development program.

Following in-clinic dosing for the initial doses (first 8 weeks) and apart from subsequent dose escalations to 20, 40, and 60 mg/day, which will be administered in the clinic, pegvaliase will typically be administered at home by the trained subject, though pegvaliase may be also administered at home by a trained caregiver or visiting healthcare provider. In addition to training in drug administration prior to the first in-clinic dose, subjects, caregivers, and adult observers will receive extensive training on how to monitor and respond to possible AEs (including acute systemic hypersensitivity reaction [anaphylaxis]) that may be associated with study drug treatment.

Pegvaliase will be administered using an I/T/M dosing regimen similar to the regimen used in Phase 3 studies performed in the US (165-301 and 165-302) and included in the USPI and SmPC (see Table 6.5.1 and Figure 1.2.2). During pegvaliase clinical development, this dosing regimen was found to help mitigate the onset and severity of hypersensitivity reactions while substantially reducing blood Phe concentrations.

The pegvaliase dose levels in this study have been evaluated in 6 multiple-dose Phase 2 and Phase 3 studies. The dose level for induction in 165-306 will be 2.5 mg once weekly and was chosen because this dose with a once weekly dosing frequency administered during the initial 4 weeks of treatment (ie, induction) used in Studies PAL-002, 165-205, and 165-301 has been associated with a lower incidence and severity of hypersensitivity reactions when compared with 5 days per week dosing frequency at a higher starting dose (0.4 mg/kg) studied in Study PAL-004.

4.4 End of Study Definition

A subject is considered to have completed the study if he/she has completed all scheduled visits, including procedures scheduled at the Study Completion/Early Termination (SC/ET) visit, as shown in the SoA (see Section 1.3), or the study is closed by the sponsor. The end of the study is defined as the date of the last visit of the last subject in the study.

5 STUDY POPULATION

Prospective approval of protocol deviations to eligibility criteria, also known as protocol exemptions, is not permitted. Rescreening is allowed for out of range laboratory values.

5.1 Inclusion Criteria

Subjects are eligible to be included in the study only if all of the following criteria apply:

Age

1. Is 12 to 17 years old, inclusive, at the start of the Screening/Run-in Period (Day -28).

Type of Participant and Disease Characteristics

2. Diagnosis of PKU and failure to maintain recommended blood Phe levels on existing management (sapropterin dihydrochloride and/or Phe-restricted diet) demonstrated by 2 blood Phe concentration measurements > 600 µmol/L during the Screening/Run-in Period (7 to 10 days in between blood Phe assessments) and average blood Phe concentration > 600 µmol/L over the past 12 months (per available data).
3. Willing and able to maintain and adjust dietary and medical protein food intake according to the study protocol.
4. If on medication for ADHD, depression, or other psychiatric disorder, stable dose of medication for ≥ 8 weeks prior to enrollment and willing to maintain stable dose unless a change is medically indicated.
5. An adult (≥ 18 years of age) has been identified who is willing and competent to observe the subject during study drug administration and for a minimum of 1 hour following administration.
6. Able and willing to comply with all study procedures.
7. Generally in good health, as evidenced by physical examination, clinical laboratory evaluations (hematology, chemistry, and urinalysis), and electrocardiogram (ECG) tests performed at Screening.

Sex and Contraceptive/Barrier Requirement

8. Males and females are eligible to participate in this clinical study.
9. Females with childbearing potential must have a negative pregnancy test at Screening/Run-in and be willing to have additional pregnancy tests during the study.
10. If sexually active, male or female subjects must not plan to become pregnant (self or partner) and must use 2 acceptable methods of contraception while participating in the study beginning at Screening and for 4 weeks after discontinuing study drug. Contraceptive use must be consistent with the guidance provided in Appendix 4, Section 10.4 of this protocol. Acceptable methods of contraception may include:

- Primary forms: hormonal (combination hormone-containing pills, patch, vaginal ring, or intrauterine device) or non-hormonal (copper-containing intrauterine device).
- Secondary forms: barrier forms and other forms of birth control and must include spermicide (eg, male condom; female condom is not an acceptable secondary form).

11. Contraceptive use should be consistent with local regulations regarding the methods of contraception for those participating in clinical studies.

Informed Consent

12. Subjects must be capable of giving signed informed consent as described in Appendix 1, Section 10.1, which includes compliance with the requirements and restrictions listed in the informed consent form (ICF) and in this protocol. In the case of subjects under the age defined by regional law or regulation, parent(s) or guardian(s) must be willing and able to provide written, signed informed consent after the nature of the study has been explained and prior to performance of any research-related procedure. Subjects under the age of majority must be willing and able to provide written assent (if required by local regulations or the IRB/IEC) after the nature of the study has been explained and prior to performance of any research-related procedure.

5.2 Exclusion Criteria

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

Medical Conditions:

1. Concurrent disease or condition that would interfere with study participation or safety (eg, history or presence of clinically significant cardiovascular, pulmonary, hepatic, renal, hematologic, gastrointestinal, endocrine, immunologic, dermatologic, neurological, oncologic, or psychiatric disease).

Prior/Concomitant Therapies:

2. Taking any of the prohibited medications outlined in Section 6.8.1.
3. Previous treatment with pegvaliase.
4. Use of any medication that is intended to treat PKU, including the use of large neutral amino acids, within 14 days prior to the administration of study drug on Day 1.
5. Use or planned use of any injectable drugs containing polyethylene glycol (PEG; other than pegvaliase), including medroxyprogesterone injection, within 3 months prior to the start of Screening/Run-in and during study participation with the exception of COVID-19 vaccinations (Section 6.8.1).
6. A history of organ transplantation or on chronic immunosuppressive therapy.

Prior/Concurrent Clinical Study Experience

7. Use of any investigational product or investigational medical device within 30 days prior to Screening/Run-in or requirement for any investigational agent prior to completion of all scheduled study assessments.

Diagnostic Assessments

8. A positive test for HIV antibody, hepatitis B surface antigen, or hepatitis C antibody.
9. Alanine aminotransferase (ALT) concentration > 2 × the upper limit of normal (ULN).
10. Creatinine > 1.5 × ULN.

Other Exclusions

11. A history of substance abuse (as defined by the American Psychiatric Association: Diagnostic and Statistical Manual of Mental Disorders [DSM]) in the past 12 months or current alcohol or drug abuse.
12. Pregnant or breastfeeding or planning to become pregnant (self or partner) or breastfeeding at any time during the study.
13. Major surgery planned during the study period.
14. Inability to identify and/or communicate to others that the subject is experiencing symptoms of potential anaphylaxis due to cognitive impairment or other reasons.
15. Unwillingness or inability of subject/caregiver to carry auto-injectable epinephrine with them at all times.
16. Any condition that, in the view of the investigator, places the subject at high risk of poor treatment compliance or terminating early from the study.

5.3 Lifestyle Considerations

5.3.1 Meals and Dietary Restrictions

Details of all PKU-specific dietary therapy (eg, Phe intake, amino-acid supplementation) must be recorded in the source document and electronic case report form (eCRF).

Dietary Instruction

Subjects must maintain dietary Phe intake levels that are consistent with their baseline levels for the duration of the study unless clinically indicated, per dietician or investigator/designee discretion, to support individual nutritional needs (refer to Section 8.1.2).

Fasting Requirements

Fasting (water only) is required for at least 2.5 hours prior to any blood Phe measurement.

Any time fasting is required for visit assessments, the fasting assessments should be completed and fasting stopped before clinical outcome assessments are performed. The fast can be broken with a snack or meal as preferred.

5.3.2 Activity Restrictions

Subjects should abstain from strenuous exercise for 48 hours before each blood collection for clinical laboratory tests. Subjects may participate in light recreational activities (such as watching television or reading) prior to study visits, as appropriate.

5.4 Screen Failures

Screen failures are defined as subjects who assent to participate (and whose parents/guardians consent to their participation) in the clinical study but who are not subsequently enrolled. A minimal set of screen failure information is required to ensure transparent reporting of screen failure subjects to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and the occurrence of any serious adverse event (SAE).

Individuals who do not meet the criteria for participation in the study may be rescreened. Rescreened subjects should be assigned the same subject number as for the initial screening.

5.5 Study Stopping Criteria

The independent Data Monitoring Committee (DMC) will be responsible for performing a benefit to risk assessment of pegvaliase in this patient population on a regular (quarterly) and ad hoc basis during the study and for making recommendations to BioMarin with regards to study conduct. The DMC Chair will be informed of all subjects meeting the Individual Stopping Criteria (Section 8.3.8.1.1).

6 STUDY TREATMENT

6.1 Study Treatment(s) Administered

Study treatment is defined as any investigational treatment(s), marketed product(s), placebo, or medical device(s) intended to be administered to a study subject according to the study protocol.

The investigational treatment is pegvaliase (recombinant *Anabaena variabilis* phenylalanine ammonia lyase-PEG). For subcutaneous administration, subjects are supplied with prefilled syringes in 3 sizes. Sizes are 2.5 mg (0.5 mL of 5 mg/mL protein concentration), 10 mg (0.5 mL of 20 mg/mL protein concentration), and 20 mg (1.0 mL of 20 mg/mL protein concentration).

The excipients in this drug product are tromethamine, tromethamine-hydrochloride, sodium chloride, trans-cinnamic acid, and water for injection.

Study treatment details are provided in Table 6.1.1.

Table 6.1.1: Study Treatment

Treatment Name	pegvaliase
Type	drug
Dose Formulation	prefilled syringe
Unit Dose Strength	Each PFS is filled to deliver at least 0.5 or 1 mL of formulation containing 2.5, 10, or 20 mg of pegvaliase
Dosage Level(s)	2.5 mg (1x or 2x per week), 10 mg (2x or 4x per week), 20 mg (daily), 40 mg (daily), 60 mg (daily)
Route of Administration	subcutaneous injection
IMP and NIMP	IMP
Sourcing	provided centrally by the sponsor
Packaging and Labeling	Drug product packaging is identified with lot and identification numbers and provided in a box labeled with the study number and identified to be for investigational use.
Current/Former Name(s) or Alias(es)	pegvaliase, BMN 165, rAvPAL-PEG (PEGylated recombinant Anabaena variabilis phenylalanine ammonia lyase), Palynziq® (trade name)

IMP, investigational medical products; NIMP, non-investigational medical products; PFS, pre-filled syringe.

In addition to pegvaliase, treatment in this study includes a diet in which total protein intake (defined as the sum of protein intake from medical food and intact food) must remain stable and consistent with baseline levels. Specifically, to accurately assess the primary endpoint comparison between the active (pegvaliase) and control (diet-only) treatment arms on the

change from baseline in blood Phe concentration following 72 weeks on study, total protein intake must remain stable and consistent with baseline levels for subjects in both treatment arms through Part 1. In Part 2, subjects in the control arm must continue to maintain this prescribed level of protein intake, whereas subjects randomized to pegvaliase whose blood Phe concentration is ≤ 360 µmol/L for a minimum of 4 weeks may adjust dietary protein intake based on individual subject response to pegvaliase and guidance from the investigator. Details on diet monitoring is provided in Section 8.1.2.

6.1.1 Additional Drug Administration Instructions

Each refilled syringe is intended for a single subcutaneous injection. The recommended injections sites include the front middle of thighs and the abdomen at least 2 inches (5 centimeters) away from the navel. If a caregiver is giving the injection, the top of the buttocks and the back of the upper arms are also appropriate injections sites. The injection sites for administration of pegvaliase should alternate between doses. Pegvaliase must not be injected into moles, scars, birthmarks, bruises, rashes, or areas where the skin is hard, tender, red, damaged, burned, inflamed, or tattooed. If multiple injections are needed for a single dose, injections should be administered at the same time of day and injection sites should be at least 5 cm away from each other. Doses should not be divided over the course of the day.

A subject (or subject-designated caregiver) must meet all of the following criteria to be eligible to administer the drug:

- Has no known cognitive impairments that may increase the safety risk per investigator assessment
- Has no medical history or current medications that may compromise administration of pegvaliase per investigator assessment
- Has completed all required drug administration training and has demonstrated administration competency per investigator assessment, including how to prepare pegvaliase for administration and safely perform the injection

Refer to Section 6.5 for study drug administration requirements regarding the presence of an adult observer and for premedication requirements.

Following a dose interruption of ≥ 4 doses, the investigator should obtain approval from the medical monitor prior to the subject restarting pegvaliase. Dose regimens may be revised provided the investigator obtains approval from the medical monitor prior to starting an alternative dose regimen.

The suitability for self-administration of pegvaliase will be reassessed by the investigator if a subject experiences any issues with self-administration. In addition, if a subject has an

NCI-CTCAE grade 3 or higher AE during the study, the subject's suitability for self-administration of pegvaliase will be reassessed by the investigator.

Between scheduled clinic visits, the clinic staff will contact the subject weekly to monitor if the subject is experiencing problems with drug administration, for any AEs, and to answer questions throughout the duration of the study. The subject must perform the scheduled assessments and procedures in the clinic as outlined in the SoA (Section 1.3). Training materials and other information specific to the study site personnel will be provided in the study reference materials, which includes drug administration training materials and instruction on the following:

- How to prepare and safely perform the injections of pegvaliase
- How to receive, store, and return both used and unused pegvaliase
- How to safely use and dispose of prefilled syringes used for injections of pegvaliase
- How to use a prefilled syringe every time drug is administered
- How to care for the injection site after an injection of pegvaliase
- How to document each pegvaliase injection
- How to identify an AE and how to report this to the study site
- How and when to use epinephrine
- Whom to contact at the study site in case of an emergency

Subjects will be provided with a workbook to document the date and time of pegvaliase injections, injection site, any suspected AEs, and other relevant information. Dosing in response to an AE should be determined by the investigator.

6.2 Preparation/Handling/Storage/Accountability

At the study site, all study drug must be stored at 5 ±3°C (41 ±5°F), under the conditions specified in the IB, and in a secure area accessible only to the designated pharmacists and clinical site personnel. All study drug must be stored and inventoried, and the inventories must be carefully and accurately documented according to applicable state, federal, and local regulations; ICH Good Clinical Practice (GCP); and study procedures. Information regarding storage of study drug in subjects' homes is provided in the study training materials.

The investigator or designee must confirm appropriate temperature conditions have been maintained during transit for all study treatment received and that any discrepancies are reported and resolved before use of the study treatment.

Only subjects enrolled in the study may receive study treatment, and only authorized site staff may supply study treatment. All study treatment must be stored in a secure, environmentally controlled and monitored (manual or automated) area in accordance with the labeled storage conditions, with access limited to the investigator and authorized site staff.

The investigator, institution, qualified pharmacist, or the head of the medical institution (where applicable) is responsible for study treatment accountability, reconciliation, and record maintenance (ie, receipt, reconciliation, and final disposition records).

Subjects are provided instructions for returning used and unused study drug prefilled syringes and for proper disposal at each site visit.

Further guidance and information for the final disposition of unused study treatments are provided in the Pharmacy Manual.

6.3 Measures to Minimize Bias: Randomization and Blinding

This is an open-label study; however, the specific intervention to be taken by a subject will be assigned using an IVRS/IWRS. The site will contact the IVRS/IWRS prior to the start of study intervention administration for each subject. The site will record the intervention assignment on the applicable case report form, if required. Potential bias will be reduced by central randomization.

6.4 Study Treatment Compliance

When subjects are dosed at the site, they will receive study treatment directly from the investigator or designee, under medical supervision. The date and time of each dose administered in the clinic will be recorded in the source documents and recorded in the eCRF. The dose of study treatment and study subject identification will be confirmed at the time of dosing by a member of the site staff other than the person administering the study treatment.

When subjects or caregivers administer study treatment(s) at home, the date, time, and quantity of each dose of study drug administered to each subject must be recorded by the subject or caregiver in a workbook provided for the study. The site staff will collect the workbook at scheduled clinic visits and transcribe this information to the appropriate eCRF.

These data will be used to assess compliance. Deviation(s) from the prescribed dosage regimen should be recorded in the eCRF. Treatment start and stop dates, including dates for treatment delays and/or dose reductions, should also be recorded in the eCRF.

6.5 Dose Modification

Subjects will receive pegvaliase at a concentration of 5.0 or 20.0 mg/mL. Pegvaliase will be administered subcutaneously at dose levels in the range of 2.5 to 60 mg. The minimum dose of pegvaliase is 2.5 mg/week. The maximum allowable daily dose of pegvaliase is 60 mg/day (for a maximum weekly total dose of 420 mg).

The duration of the Primary Treatment Phase (Part 1) is 72 weeks, during which pegvaliase treatment will be initiated for subjects randomized to the active (pegvaliase) treatment arm using an I/T/M dosing regimen. The recommended dosing schedule for subjects dosing with pegvaliase is shown in Table 6.5.1. Induction will consist of a 4-week period when subjects randomized to the active treatment arm will receive pegvaliase subcutaneously with a PFS at a fixed dose of 2.5 mg/week and during which the dosing regimen will not be modified in response to blood Phe concentration. Once subjects complete induction they will titrate up to a dose of 10 mg/day. The dosing frequency will be increased gradually during titration dosing to daily (7 days/week). During Maintenance Dosing the dose will be increased to 20 mg/day, 40 mg/day, and 60 mg/day depending upon individual blood Phe lowering efficacy (per the investigator's discretion). The dose should be increased to 40 mg/day if blood Phe is > 360 µmol/L after 24 weeks dosing at 20 mg/day and increased to 60 mg/day if blood Phe is > 360 µmol/L after 16 weeks dosing at 40 mg/day. BioMarin recommends that the target blood Phe levels should be based on individual needs, with a target blood Phe ≤ 600 µmol/L.

Duration between dose titration steps may be increased in response to AEs. Dose level reductions due to AEs or hypophenylalaninemia (confirmed with 2 consecutive blood Phe values < 30 µmol/L) may be made at any time during the study at the discretion of the investigator. Phe levels will be regularly monitored and adjustments in the pegvaliase dose and/or dietary intake of Phe will be required to mitigate the potential risk of chronic hypophenylalaninemia levels (< 30 µmol/L) in adolescent subjects.

Following completion of Part 1, subjects randomized to the active (pegvaliase) treatment arm will enter the Extension Phase (Part 2), during which subjects will continue dosing with pegvaliase at their optimal maintenance dose level for up to an additional 80 weeks in order to evaluate the long-term safety and efficacy of pegvaliase in adolescent subjects with PKU.

Following completion of Part 1, subjects randomized to the control (diet-only) treatment arm in Part 1 will repeat the Part 1 assessment schedule while initiating pegvaliase dosing from Weeks 73 through 145, after which they will receive pegvaliase for up to 8 additional weeks (through Week 153).

Table 6.5.1: Study Drug Dose Titration Schedule

Part 1 Dosing	Duration [a]	Dose (mg)	Frequency of Administration (Doses Per Week)
Induction	4 weeks	2.5	1
Titration	5 weeks	2.5	2
		10	1
		10	2
		10	4
		10	7
Maintenance	20 mg daily: 24 weeks [b] 40 mg daily: 16 weeks [b,c] 60 mg daily: maximum dose [c]	20	7
		40	7
		60	7

[a] Minimum duration prior to dose escalation. Dose escalation may be delayed depending on subject tolerability.

[b] Prior to initial dose escalation, the minimum amount of time at 20 mg/day is 24 weeks and the minimum amount of time at 40 mg/day is 16 weeks. The dose should be increased to 40 mg/day if blood Phe is > 360 µmol/L after 24 weeks dosing at 20 mg/day, and increased to 60 mg/day if blood Phe is > 360 µmol/L after 16 weeks dosing at 40 mg/day.

[c] If multiple injections are needed for a single dose, the injections should be administered at the same time of day and injection sites should be at least 5 cm away from each other. Doses should not be divided over the course of the day.

Following enrollment, subjects (and an adult caregiver) will be trained on how to administer study drug at home. Pegvaliase dosing will be conducted in-clinic for at least the first 8 weeks including titration up to 10 mg once daily, and for all subsequent dose escalations. The subject (and the adult caregiver) must demonstrate competency with injecting study drug in order to be permitted to administer study drug at home. Dosing at home will not be allowed until the principal investigator has assessed the subject's and adult observer's competency in proper study drug administration and recognition and appropriate management of the potential risks.

Throughout the study, an adult observer must be present during study drug administration and for a minimum of 1 hour following administration. An adult observer is defined as someone who will be present with the subject during and after pegvaliase administration, is able to recognize the signs and symptoms of an anaphylaxis episode, and is able to call for emergency medical support and administer epinephrine, if warranted. Administration of study drug may only be performed if this adult observer is present. Subjects and the adult observers who are observing them during study drug administration will be provided with information and extensive training regarding how to recognize a possible allergic reaction, the severity of the reaction, and instructions on what to do if a reaction occurs. Home

administration may not performed until the investigator has assessed the adult observer's competency in proper study drug administration and the recognition and appropriate management of potential risks.

Subjects will be provided with 2 epinephrine injectors and are required to carry both injectors with them at all times. Subjects and adult observers will be trained to recognize the signs and symptoms of anaphylaxis and, if these reactions occur, to call for emergency medical support and administer an epinephrine injector. For the duration of the study, each subject will be contacted weekly by study site personnel to monitor for administration problems and AEs (see Section 8.3.8.1 for details on the management of suspected anaphylaxis in this study).

All subjects receiving pegvaliase must be premedicated with an H1 antagonist, and an H2 antagonist, and an antipyretic approximately 2 to 3 hours prior to each dose until they reach the Maintenance Phase, defined as when subjects achieve blood Phe ≤600 µmol/L while they are on a stable dose of pegvaliase. Premedication may be considered during the Maintenance Phase at the investigator's discretion, eg, approximately 2 to 3 hours prior to study drug for 1 week upon reintroduction of study drug, upon resolution of an AE, following any dose interruption of ≥ 4 days, and for any dose increases in the Extension Phase. Subjects may also be premedicated at any other time in the study per investigator discretion.

This premedication regimen was successfully implemented in the Phase 3 adult clinical program. Subjects will be provided with a workbook to document the date and time of study drug injections, the injection site, premedication use, and suspected AEs.

If ≥ 4 consecutive doses are missed for reasons other than safety related issues, the investigator should obtain approval from the medical monitor prior to the subject resuming study drug.

6.5.1 Dosing Modifications in Response to Hypersensitivity Adverse Events

Dosing modifications in response to a hypersensitivity adverse event (HAE) depends on the NCI-CTCAE grade and suspected relationship study drug. Dosing instructions are provided in Table 6.5.1.1 (regardless of previous occurrence). Dosing modifications are per reported HAE, independent of prior reported HAEs.

Table 6.5.1.1: Dosing Instructions in Response to a Hypersensitivity Adverse Event

NCI-CTCAE Grade [a]	Related to Study Drug	Action with Study Drug			Individual Stopping Criteria Evaluation [d]	HRV Assessment [e]
		Maintain [b]	Reduce [c]	Interrupt [c]		
1	Yes or No	X	(X) Optional	(X) Optional		Investigator discretion
2	Yes or No	X	(X) Optional	(X) Optional		Investigator discretion
3	No				X Immediately consult with sponsor medical monitor	Investigator discretion
3 [d]	Yes			X	X Immediately consult with sponsor medical monitor	Yes (if within 24 hours of onset)
4 [d]	Yes or No			X	X Immediately consult with sponsor medical monitor	Yes (if within 24 hours of onset)

AE, adverse event; CTCAE, Common Terminology Criteria for Adverse Events, Version 5.0; HRV, Hypersensitivity Reaction Visit; NCI, National Cancer Institute.

[a] NCI-CTCAE grade determination is performed by the investigator and may be done either via telephone or clinic visit.

[b] The investigator will instruct the subject to maintain the study drug dose at the time of AE onset until improvement to Grade 1 or resolution (per investigator assessment in the clinic or via telephone).

[c] The study drug dose may be reduced or interrupted if necessary per investigator determination.

[d] If a subject has an NCI-CTCAE Grade ≥ 3 hypersensitivity AE that is related to study drug and is suspected to meet Brown's criteria for severe (Grade 3) in the judgment of the investigator and/or the sponsor's medical monitor, the subject will be permanently discontinued from study drug.

[e] If the investigator determines that the NCI-CTCAE Grade ≥ 3 hypersensitivity reaction is related to administration with study drug, the subject will be asked to return to the clinic within 24 hours of event onset for evaluation, including laboratory tests (chemistry, hematology, urinalysis, anti-pegvaliase IgE [sampling should be performed 8 to 24 hours after event onset and before the next dose of study drug], urine albumin/creatinine ratio, CRP, C3, and C4).

Once an HAE (other than an anaphylaxis episode) improves to Grade 1 or resolves, the study drug dose may be increased, maintained, or reduced, at the discretion of the investigator. If reduced, reductions should be from 60 to 40 mg/day, from 40 to 20 mg/day, or from 20 to 10 mg/day, whichever is appropriate.

6.6 Treatment after the End of the Study

There is no treatment following completion of the study.

6.7 Overdose

For this study, any dose of pegvaliase greater than the prescribed dose will be considered an overdose.

BioMarin does not recommend specific treatment for an overdose. In the event of an overdose, the investigator should contact the BioMarin medical monitor immediately.

6.8 Concomitant Therapy

Any medication or vaccine (including over-the-counter or prescription medicines, vitamins, and/or herbal supplements) that the subject is receiving at the time of enrollment or receives during the study must be recorded on the designated eCRF along with:

- Reason for use
- Dates of administration including start and end dates
- Dosage information including dose and frequency

The medical monitor should be contacted if there are any questions regarding concomitant or prior therapy. The investigator may prescribe additional medications, deemed necessary to provide adequate prophylactic or supportive care, during the study, as long as the prescribed medication is not prohibited by the protocol (refer to Section 5.2). In the event of an emergency, any needed medications may be prescribed without prior approval, but the medical monitor must be notified of the use of any contraindicated medications immediately thereafter. Any concomitant medications added or discontinued during the study should be recorded on the eCRF. Medications should, whenever possible, not be recorded in the electronic database with a frequency of PRN.

6.8.1 Excluded Concomitant Medications

The following medications are prohibited starting at the time specified and until the end of the study:

- Any medication that is intended to treat PKU, including the use of large neutral amino acids, within 14 days prior to the administration of study drug on Day 1.
- Any injectable drugs containing polyethylene glycol (PEG; other than pegvaliase), including medroxyprogesterone injection, within 3 months prior to the start of Screening/Run-in and during study participation with the exception of COVID-19 vaccinations. Please contact the medical monitor prior to administering COVID-19 vaccinations.
- Use of any investigational product or investigational medical device within 30 days prior to Screening/Run-in or requirement for any investigational agent prior to completion of all scheduled study assessments.

In addition, if a subject is on medication for ADHD, depression, or other psychiatric disorder, the dose must be stable for ≥ 8 weeks prior to enrollment and must continue to be stable throughout the study unless a change is medically indicated.

6.8.2 Permitted and/or Required Concomitant Medications

All prescription and over-the-counter medication including herbal supplements and medical food taken by a subject for 30 days before screening are recorded on the designated CRF. Psychiatric medications and their dosage should remain consistent throughout the study unless a change is medically indicated. No new psychiatric medication should be initiated during the study unless medically indicated. No psychotherapy should be initiated during the study unless medically indicated. The investigator may prescribe additional medications during the study as long as the prescribed medication is not prohibited by the protocol (see Section 6.8.1). In the event of an emergency, any needed medications may be prescribed without prior approval but the medical monitor must be notified of the use of any contraindicated medication immediately thereafter. Any concomitant medication added or discontinued during the study should be recorded on the appropriate CRF.

All subjects receiving pegvaliase will be required to premedicate with an H1 antagonist, and an H2 antagonist, and an antipyretic approximately 2 to 3 hours prior to each dose of study drug until they reach the Maintenance Phase, defined as when subjects achieve blood Phe ≤600 µmol/L while they are on a stable dose of pegvaliase. Premedication may be considered during the Maintenance Phase at the investigator's discretion. For further information regarding premedication, refer to Section 6.5.

7 DISCONTINUATION OF STUDY TREATMENT AND SUBJECT WITHDRAWAL FROM STUDY

7.1 Discontinuation of Study Treatment

In rare instances it may be necessary for a patient to permanently discontinue study treatment. If study treatment is permanently discontinued, the subject will be asked to continue to provide consent at the time of treatment discontinuation to remain in the study and undergo the scheduled study assessments. Refer to the SoA for data to be collected at the time of discontinuation of study treatment and follow-up, and for any further evaluations that need to be completed.

If the subject declines to provide consent to remain in the study, the study completion visit assessments should be performed as detailed in the SoA (Section 1.3).

7.2 Subject Withdrawal from the Study

A subject may withdraw from the study at any time at his or her own request, or may be withdrawn at any time at the discretion of the investigator for safety, behavioral, compliance, or administrative reasons.

At the time of discontinuing from the study, if possible, an early termination (ET) visit should be conducted, as shown in the SoA (Section 1.3).

The subject will be permanently discontinued both from the study treatment and from the study at that time.

If the subject withdraws consent for disclosure of future information, the sponsor may retain and continue to use any data collected before such a withdrawal of consent.

If a subject withdraws from the study, BioMarin will only use the remaining samples to complete study procedures as described in Section 8 and no new samples will be collected. If a subject no longer wants the remaining samples to be used for future research, he/she shall inform the study investigator or institution accordingly and BioMarin will only use the remaining samples for health authority requests related to the activities in Section 8, not for future research. The investigator must document this in the site study records.

7.3 Lost to Follow-Up

A subject will be considered lost to follow-up if he or she repeatedly fails to return for scheduled visits and cannot be contacted by the site.

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

- The site must attempt to contact the subject to reschedule the missed visit as soon as possible, counsel the subject on the importance of maintaining the assigned visit schedule, and ascertain whether or not the subject wishes to and/or should continue in the study.
- Before a subject is deemed lost to follow up, the investigator or designee must make every effort to regain contact with the subject (where possible, 3 telephone calls and, if necessary, a certified letter to the subject's last known mailing address or local equivalent methods). These contact attempts should be documented in the subject's medical record.
- Should the subject continue to be unreachable, he or she will be considered to have withdrawn from the study.

Discontinuation of specific sites or of the study as a whole are handled as part of Appendix 1, Section 10.1.9.

8 STUDY ASSESSMENTS AND PROCEDURES

An ICF must be signed and dated by the subject's legally authorized representative, the investigator or designee, and a witness (if required) before any study-related procedures are performed.

All screening evaluations must be completed and reviewed to confirm that potential subjects meet all eligibility criteria. The investigator will maintain a screening log to record details of all subjects screened and to confirm eligibility or record reasons for screening failure, as applicable. Procedures conducted as part of the subject's routine clinical management (eg, blood count) and obtained before signing of the ICF may be utilized for screening or baseline purposes provided the procedures met the protocol-specified criteria and were performed within the time frame defined in the SoA.

Potential subjects will enter the Screening/Run-in period, which will last 4 weeks and include 2 assessments of blood Phe concentration 7 to 10 days apart. Diet diaries should be completed for the 3 days preceding each of the blood Phe assessments. Following confirmation that the 2 blood Phe results are > 600 µmol/L and all other eligibility criteria have been met, subjects may be enrolled in the study on Day 1. Protocol exemptions for eligibility are not allowed. Following enrollment, subjects will be randomized 2:1 to the active (pegvaliase) or control (diet-only) treatment arm of the study.

In the Primary Treatment Phase (Part 1), subjects in the active (pegvaliase) and control (diet-only) treatment arms will follow the same visit schedules and perform the same assessments except that the control subjects will not be receiving pegvaliase so will not have PK draws or immunogenicity assessments. The timing of Part 1 assessments for active and control subjects is provided in Table 1.3.1 and Table 1.3.3, respectively.

The primary efficacy endpoint, the change from baseline blood Phe, is assessed following 72 weeks on study (the end of Part 1). Beginning Week 73, subjects in the active treatment arm will continue to receive pegvaliase in the Extension Phase (Part 2) for up to an additional 80 weeks (through Week 153). The timing of Part 2 assessments for active treatment subjects is provided in Table 1.3.2.

Subjects in the control (diet-only) treatment arm will initiate pegvaliase treatment beginning Week 73 and, from Weeks 73 through 145, will follow the same dosing and assessment schedule that the active subjects followed from Weeks 1 through 73, after which they will receive pegvaliase for 8 additional weeks (through Week 153). The timing of Part 2 assessments for control subjects is provided in Table 1.3.4. All the assessments, dietary rules, and other restrictions that applied to active subjects in the Primary Treatment Phase (Part 1)

apply to control subjects in Part 2 until the Week 145 visit when change from baseline blood Phe for these subjects will be assessed after 73 weeks of pegvaliase treatment.

The last scheduled visit, which includes study drug administration, is at Week 153 for subjects randomized to both the active (pegvaliase) and control (diet-only) treatment arms. Subjects should perform the Study Completion (SC) Visit 4 weeks after the last dose of study drug or after completion of study participation, whichever occurs last. For subjects who terminate from the study early, the Early Termination (ET) visit should occur within 4 weeks after the last study drug dose or after withdrawal from study participation, whichever occurs last. If study drug is discontinued before study completion, the investigator will ask the subject to remain in the study to continue study visits and assessments, provided the subject's health, safety, and welfare would not be detrimentally affected.

Immediate safety concerns should be discussed with the sponsor (medical monitor) immediately upon occurrence or awareness to determine if the subject should continue or discontinue study treatment.

Adherence to the study design requirements, including those specified in the SoA, is essential and required for study conduct.

8.1 Efficacy Assessments

Planned timepoints for all efficacy assessments are provided in the SoA (refer to Section 1.3).

8.1.1 Blood Phe Level

The primary efficacy endpoint is change in blood Phe concentration from treatment naïve baseline following 72 weeks on study (see Section 9.4.2). Blood samples for blood Phe analysis are to be drawn according to the SoA (see Section 1.3). Monitoring of blood Phe levels is the cornerstone of diagnosis and treatment of PKU patients. Guidelines for the treatment of PKU are based on blood Phe levels (Vockley 2014; van Wegberg 2017), and clinicians and patients use blood Phe concentration to assess and monitor PKU disease progression and control.

Subjects must fast for at least 2.5 hours prior to blood Phe assessments to lessen the effect of food intake on blood Phe concentration. Samples for blood Phe concentration will be analyzed by a central laboratory.

8.1.2 Total Dietary Protein Intake, Dietary Monitoring, and Dietary Protocol Restrictions

Protein from medical foods (Phe-free amino acid fortified food sources) and protein from intact foods (any other food sources containing Phe) are collectively referred to as total dietary protein. Although total dietary protein intake is not an efficacy endpoint in this study, it is an important outcome measure that must remain stable for the primary endpoint to be accurately assessed. Specifically, to accurately assess the primary endpoint comparison between the active (pegvaliase) and control (diet-only) treatment arms on the change from baseline in blood Phe concentration following 72 weeks on study, total protein intake must remain stable for subjects in both treatment arms during this period. It is essential that subjects maintain stable protein intake to ensure that the measured changes in their blood Phe are attributable to study treatment rather than changes in their dietary protein intake.

A subject's diet is monitored using a 3-day diet diary. The 3-day dietary record (diet diary) is completed by subjects, brought to clinic visits for review, and maintained with the study source documents. Subjects record all food, beverages, special low-protein foods, and medical foods consumed for 3 consecutive days prior to the scheduled clinic visit and data are analyzed by the nutritional software (Metabolic Pro®) for total kcals, protein, Phe, tyrosine, and the percentage of daily recommended intake provided for protein, Phe, tyrosine, vitamins, and minerals.

A dietitian under investigator supervision is recommended to monitor and manage the subject's diet for the entire duration of the study, though an investigator or designee may also perform this role. Subjects will be provided with 3-day diaries in which all dietary protein intake (including medical food and/or intact food) must be recorded for 3 consecutive days immediately prior to each scheduled clinic visit for review with the dietitian. The subject may be asked to repeat the 3-day diary collection if the dietitian or investigator/designee considers the diary has not been completed accurately; in such circumstances, study visits will be rescheduled. All subjects will be provided the option of tyrosine supplementation (500 mg, 3 times per day with meals) at the discretion of the investigator.

The change from baseline in total protein intake at Week 73 will be compared as a secondary endpoint. For this endpoint, baseline protein intake is defined as the average of protein intake values derived from diet diaries collected during Screening/Run-in. During Screening/Run-in, 3-day diet diaries are completed 3 times, each time covering the 3 days periods prior to blood Phe assessments (and collected on the day of those assessments). The 3 Screening/Run-in blood Phe assessments are scheduled to occur (1) at Day -25 to Day -23; (2) 7 to 10 days later (ie, in the Day -18 to Day -13 window); and (3) at Day 1 pre-dose.

Thus, the 3 day diet assessments will assess diet for (1) the 3 days within the Day -28 to Day -23 window immediately prior to the first blood Phe assessment; (2) the 3 days within the Day -21 to Day -15 window immediately prior to the second blood Phe assessment; and (3) for Days -3 to Day 1.

Subjects will be instructed not to change their total dietary protein intake (medical food and/or intact food) during the Screening/Run-in and Primary Treatment Phase (Part 1) of the study. However, modifications to a subject's diet and reductions in study drug dose level must be implemented if blood Phe levels are reduced to < 30 µmol/L and confirmed upon recheck (performed within approximately 2 weeks) (see Section 8.1.2.1).

In Part 2, dietary protein intake may be modified for subjects randomized to active (pegvaliase) treatment if blood Phe concentration is ≤ 360 µmol/L for a minimum of 4 weeks. Should that occur, subjects may adjust dietary protein intake based on individual subject response to pegvaliase and guidance from the investigator or designee (see Section 8.1.2.2). Subjects randomized to the control (diet-only) treatment arm must continue to maintain dietary protein intake consistent with baseline through Week 145 except in the case of blood Phe levels < 30 µmol/L confirmed upon recheck (see Section 8.1.2.1).

Baseline total dietary protein intake will be used as the comparator for subsequent 3-day diet diary entries. Subjects will be required to maintain dietary protein intake levels that are consistent with their baseline levels for the entire duration of the study, except as otherwise specified above, with a consistent diet defined as one in which the intact protein changes are < 15% from baseline and the medical food protein changes are < 15% from baseline.

If a subject demonstrates ≥ 15% change from baseline intake of total intact protein and/or medical food protein, the dietitian (or investigator/designee) will counsel the subject to resume their baseline diet and may perform another 3-day diet record and blood Phe concentration assessment in 2 weeks. If the subject demonstrates ≥ 15% increase in intake of protein from intact foods and/or medical food at the next scheduled study visit, a discussion with the medical monitor may be required to discuss further actions related to non-adherence with consistent dietary protein intake throughout the study, at the discretion of the investigator.

If dietary protein intake is determined to be inconsistent with baseline values, it is recommended that the study site dietitian (or investigator/designee) counsel the subject on how to return to baseline values by modifying intake of protein from intact foods and/or medical food. If diet changes have been implemented, a blood Phe concentration assessment and 3-day diet record should be collected 2 weeks after implementation, in addition to all

regularly scheduled assessments. Any changes must also be documented on the appropriate eCRF.

8.1.2.1 Diet Modification for Blood Phe Levels Less than 30 µmol/L

In addition to protocol required dose reductions (Section 6.5), modifications to subject's diet should be performed if blood Phe levels are < 30 µmol/L and confirmed upon repeat blood Phe measurement (performed within approximately 2 weeks). Subjects will receive counseling for modification of intake of intact protein and medical foods based on intake at the time of blood Phe assessment, as follows:

- If it is determined that a subject is consuming < 2x their recommended level of intact protein per Dietary Reference Intake (DRI) guidelines (1.6 g/kg/day), the dietitian may instruct the subject to increase their intact protein by 20 grams/day and decrease their medical food protein by 20 grams/day.
- If it is determined that a subject is consuming ≥ 2x their recommended level of intact protein per DRI guidelines, the dietitian may instruct the subject to increase their intact protein by 10 grams/day and decrease their medical food protein by 10 grams/day.

Further increases in protein intake as per above are allowed if blood Phe levels are < 30 µmol/L in order to achieve 2 x DRI guidelines (1.6 g/kg/day) of intact protein intake. This increased intake will now be the new reference baseline for dietary protein intake for future dietary assessments. Medical food may be discontinued once the dietitian determines that the essential amino acids meet the appropriate levels for age.

8.1.2.2 Dietary Changes for Blood Phe ≤360 µmol/L in Extension Phase (Subjects Randomized to Pegvaliase)

Once subjects initially randomized to active (pegvaliase) treatment enter the Extension Phase (Part 2) of the study, dietary protein intake may be modified if blood Phe concentration reaches ≤ 360 µmol/L for a minimum of 4 weeks. If diet is modified, it is recommended to increase protein from intact foods in 10 or 20 gram increments depending on intact protein intake, and decrease protein from medical food by a similar amount, 10 or 20 grams unless the subject is already consuming the appropriate protein levels for age (World Health Organization 2007). Medical food may be discontinued once the dietitian or investigator/ designee determines that the essential amino acids provided from intact protein foods meet the appropriate levels for age.

From the beginning of the Extension Phase (Part 2), subjects initially randomized to active (pegvaliase) treatment who have achieved blood Phe > 30 to ≤360 µmol/L may have their medical food intake reduced or discontinued and intact protein intake increased, in

accordance with Table 8.1.2.2.1. Implementation of dietary adjustments will be performed in consultation with the site dietitian.

Table 8.1.2.2.1: Dietary Adjustment Algorithm in Part 2 for Subjects Randomized to Active (Pegvaliase) Treatment

Phe Category [a]	Protein Intake from Intact Food Sources [b]	Action	
		Protein Intake from Intact Food Sources	Protein Intake from Medical Food
> 30 [d] to ≤ 360 µmol/L	> 2 × RDI (> 1.7 g/kg)	Maintain or reduce by 10 g/day [c]	Discontinue [c]
	> 1 × to 2 × RDI (> 0.8 to 1.7 g/kg)	Maintain	Discontinue [c]
	0.5 × to 1 × RDI (0.4 to 0.8 g/kg)	Increase 10 g/day [c]	Decrease 10 g/day [c]
	< 0.5 × RDI (< 0.4 g/kg)	Increase 20 g/day [c]	Decrease 20 g/day [c]

RDI, recommended daily intake.

[a] Based on results of the previous blood Phe assessment; blood Phe reductions may not be observed until the subject is titrated to an effective dose.

[b] RDI (0.83 g/kg) is based on the joint FAO/WHO/UNU Expert Consultation on Protein and Amino Acid Requirements in Human Nutrition (World Health Organization 2007).

[c] The resulting adjusted dietary protein intake will become the new reference baseline for dietary protein intake for future dietary assessments.

[d] If a subject experiences blood Phe levels < 30 µmol/L on two consecutive blood tests, refer to the action to take in the event of blood Phe ≤ 30 µmol/L (Section 8.1.2.1).

8.1.3 Exploratory Assessments

8.1.3.1 Neurocognitive Assessments

The ADHD-RS IV and BRIEF instruments will be used to evaluate the effect of pegvaliase treatment on neurocognitive outcomes in adolescent subjects with PKU. The same parent/guardian must complete both the ADHD-RS IV and the BRIEF at all scheduled time points.

8.1.3.1.1 The ADHD Rating Scale IV Inattention Subscale (ADHD-RS)

The ADHD-RS IV (American Psychiatric Association 2013; DuPaul 1998) will be used to assess inattention and hyperactivity symptoms among the adolescents enrolled in this study. The ADHD-RS is derived directly from diagnostic criteria delineated in the DSM, is well validated, and has been extensively used in a broad array of disease classes, including PKU (Gassio 2010; Wyrwich 2016). The ADHD-RS IV was the secondary endpoint in the

BioMarin Phase 3 pegvaliase study (165-302) in which improvement in inattention symptoms was associated with reduction in mean blood Phe levels (Thomas 2018).

The ADHD-RS IV instrument is completed independently by the parent/guardian. The same parent/guardian must complete the scale at each scheduled time point. The instrument consists of 2 nine-item scales that evaluate symptom severity for inattention and hyperactivity/impulsivity, respectively, during the previous month. Each scale is scored separately. Each item is rated on a 4-point severity scale (0, none; 1, mild; 2, moderate; 3, severe); scores of 2 or 3 on an individual item indicate a symptom. The PKU literature suggests that inattentive symptoms are more prevalent than hyperactivity and impulsivity symptoms among individuals with PKU; thus, the inattentive sub-scale is considered the most relevant and will be included in analyses (Bilder 2016).

A vendor specializing in cognitive testing provides study staff training for the investigator-rated ADHD-RS IV administration.

8.1.3.1.2 Behavior Rating Inventory of Executive Function (BRIEF)

The BRIEF is an 86-item measure designed to assess executive function skills in children 5 to 18 years of age (Gioia 2002). The BRIEF was developed to address the limitations of available assessments in examining real-word expressions of behaviors related to executive function. The BRIEF is intended to provide a standard method of asking multiple raters about executive function in daily life in a manner that is not specific to any particular disease. Because it is non-disorder-specific, BRIEF assesses executive function behaviors in children and adolescents experiencing a wide range of difficulties, such as those related to learning, attention, brain injuries, development disorders, and various psychiatric conditions and medical issues. The BRIEF is most often used to assess attention deficit hyperactivity disorder.

The BRIEF instrument (completed by the parent/guardian) contains 86 items in 8 non-overlapping clinical scales and 2 validity scales. The same parent/guardian must complete the BRIEF at each scheduled time point. These scales form 2 indexes: (1) Behavior Regulation (3 scales) and (2) Metacognition (5 scales), as well as a Global Executive Composite score which takes into account all of the clinical scales and represents the adolescent's overall executive function.

8.2 Safety Assessments

Planned time points for all safety assessments are provided in the SoA (Section 1.3).

8.2.1 Physical Examination

Physical examinations (full or brief) should be performed at the timepoints indicated in the SoA (Section 1.3):

- A full physical examination will include, at a minimum, general appearance (head, eyes, ears, nose, and throat) and an assessment of cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, musculoskeletal, and neurologic systems.
- A brief physical examination may be performed at the discretion of the investigator based on the subject's clinical condition and may include, but is not limited to, general appearance and an assessment of cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, musculoskeletal, and neurologic systems.

Investigators should pay special attention to clinical signs related to previous serious illnesses.

Height and weight will also be measured and recorded at the timepoints indicated in the SoA (Section 1.3).

8.2.2 Vital Signs

Vital signs include respiration rate, temperature, and single measurements of heart rate and seated diastolic and diastolic blood pressure. Any clinically significant change in vital signs will be recorded as an AE. Blood pressure and pulse measurements should be preceded by at least 5 minutes of rest for the subject in a quiet setting without distractions (eg, television, cell phones). Blood pressure and pulse measurements will be assessed with a completely automated device. Manual techniques will be used only if an automated device is not available.

Results will be recorded on the eCRF. Any abnormal vital sign assessments should be repeated once, and repeat value should be recorded in the eCRF.

8.2.3 Electrocardiograms

Electrocardiograms (ECGs) will be performed at Screening, Baseline, and SC/ET, as shown in the SoA (Section 1.3). Single 12-lead ECG will be obtained using an ECG machine that automatically calculates the heart rate and measures PR, QRS, QT, and QTc intervals.

8.2.4 Clinical Safety Laboratory Assessments

See Appendix 2, Section 10.2 for the table of clinical laboratory tests to be performed and refer to the SoA (Section 1.3) for the timing and frequency.

The investigator must review the laboratory report and document this review. The laboratory reports must be filed with the source documents. If known, the underlying diagnosis associated

with abnormal clinical laboratory test results that are considered clinically significant by the investigator should be recorded on the AE eCRF. Clinically significant abnormal laboratory findings are those which are not associated with the underlying disease, unless judged by the investigator to be more severe than expected for the subject's condition.

All abnormal laboratory tests with values considered clinically significant per the investigator during participation in the study and 30 days after the last dose of study treatment should be repeated until the values return to normal or baseline or are no longer considered clinically significant by the investigator.

In the event of elevated urinary protein on a test result, a repeat urinalysis should be performed. This repeat urine sample must be performed in the morning at the first or second morning void to allow for accurate test results and may be performed by a home healthcare nurse. Subjects with a confirmed urine/albumin creatinine ratio of ≥ 100 mg/g should be referred to a nephrologist for consultation if results were within normal range at Baseline. Subjects who had elevated results at Baseline followed by a confirmed subsequent increase of ≥ 100 mg/g from Baseline should also be referred to a nephrologist for consultation.

If clinically significant values do not return to normal/baseline within a period of time judged reasonable by the investigator, the etiology should be identified and the medical monitor notified.

All protocol-required laboratory assessments, as defined in Appendix 2, Section 10.2, must be conducted in accordance with the laboratory manual and the SoA.

If laboratory values from non-protocol specified laboratory assessments performed at the institution's local laboratory require a change in subject management or are considered clinically significant by the investigator (eg, SAE or AE or dose modification), then the results must be recorded in the eCRF.

8.2.5 Pregnancy Testing

Based on findings in studies of pregnant animals without PKU treated with pegvaliase-pqpz, pegvaliase may cause fetal harm when administered to a pregnant woman. Because the risks of taking study drug during pregnancy and breastfeeding in humans are unknown, subjects must not take study drug if they are trying to conceive, are pregnant, or are breastfeeding. From Day –28 until the end of the study, sexually active subjects must use 2 acceptable methods of contraception as outlined in the inclusion criteria section of this protocol (Section 5.1). Subjects who are confirmed to be pregnant by a serum pregnancy test and are temporarily off study drug are not required to perform the scheduled urine pregnancy tests.

In addition to contraception requirements, females of child-bearing potential are required to have urine pregnancy tests performed at the timepoints specified in the SoA (Section 1.3). Urine pregnancy test should be performed by the local laboratory. A serum pregnancy test will be performed by the central laboratory in the event of a positive or equivocal urine pregnancy test result.

Refer to Section 8.3.5 for details on the reporting procedures in the event of pregnancy.

8.3 Adverse Events and Serious Adverse Events

The definitions of an AE or SAE can be found in Appendix 3, Section 10.3.

AEs will be reported by the subject (or, when appropriate, by a caregiver, surrogate, or the subject's legally authorized representative).

The investigator and any medically qualified designees are responsible for identifying, documenting, and recording events that meet the definition of an AE or SAE and remain responsible for following up AEs that are serious, considered related to the study treatment or study procedures, or that caused the subject to discontinue the study treatment (refer to Section 7).

8.3.1 Time Period and Frequency for Collecting AE and SAE Information

Control (diet-only) treatment arm subject reporting:

- After informed consent to Day 1, only SAEs related to protocol mandated procedures will be collected.
- All AEs, SAEs, AESI, pregnancy and device related events will be collected from Day 1 through 4 weeks after last dose of study treatment or the study completion visit, whichever is longer.
- Non serious events that occur after informed consent but prior to Day 1 will be recorded on the Medical History/Concurrent Medical Conditions eCRF, not the AE eCRF.

Active (pegvaliase) treatment arm subject reporting:

- After informed consent through the first dose of study treatment, only SAEs related to protocol mandated procedures will be collected.
- All AEs, SAEs, AESI, pregnancy, and device-related events will be collected after the first dosed of study treatment through 4 weeks after the last dose of study treatment or the study completion visit, whichever is longer.
- Non serious events that begin after informed consent but before study treatment will be recorded on the Medical History/Concurrent Medical Conditions eCRF, not the AE eCRF.

All SAEs and AESIs must be recorded and reported to the sponsor or designee immediately; under no circumstances should this exceed 24 hours after the site becomes aware of the event, as indicated in Appendix 3, Section 10.3. The investigator must submit any updated SAE data to the sponsor within 24 hours of it being available.

Investigators are not obligated to actively seek AE or SAE information after conclusion of study participation. However, if the investigator learns of any SAE, including a death, at any time after a subject has been discharged from the study and he/she considers the event to be reasonably likely to be related to the study treatment or study participation, the investigator must promptly notify the sponsor.

8.3.2 Method of Identifying AEs and SAEs

The method of recording, evaluating, and assessing causality of AEs and SAEs and the procedures for completing and transmitting SAE reports are provided in Appendix 3, Section 10.3.

Care will be taken not to introduce bias when identifying AEs and/or SAEs. Open-ended and non-leading verbal questioning of the subject is the preferred method to inquire about AE occurrences.

8.3.3 Follow-up of AEs and SAEs

After the initial AE/SAE report, the investigator is required to proactively follow each subject at subsequent visits/contacts. All SAEs and AESIs (as defined in Section 8.3.8) will be followed until resolution, stabilization, the event is otherwise explained (ie, not related to study drug and/or study participation), or the subject is lost to follow-up (as defined in Section 7.3). Further information on follow-up procedures is provided in Appendix 3, Section 10.3.

8.3.4 Regulatory Reporting Requirements for SAEs

Prompt notification by the investigator to the sponsor of a SAE is essential so that legal obligations and ethical responsibilities towards the safety of subjects and the safety of a study treatment under clinical investigation can be met.

The sponsor has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a study treatment under clinical investigation. The sponsor will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority, Institutional Review Boards (IRB)/Independent Ethics Committees (IEC), and investigators.

Expedited safety reports must be prepared for suspected unexpected serious adverse reactions according to global regulatory requirements and sponsor policy and forwarded to investigators.

An investigator who receives an expedited safety report or other specific safety information from the sponsor will review and then file it along with the IB and will notify the IRB/IEC as appropriate.

8.3.5 Pregnancy

The importance of pregnancy prevention for study subjects and their partners will be communicated to all study subjects with regular reminders during the study.

Any female subject who becomes pregnant while participating in the study will temporarily discontinue study treatment.

Details of all pregnancies in female subjects and, if indicated, female partners of male subjects, will be collected after the start of study treatment and until 4 weeks after the last dose of study treatment.

While pregnancy itself is not considered to be an AE or an SAE, any pregnancy complication or elective termination of a pregnancy for medical reasons will be reported as an AE or SAE.

Abnormal pregnancy outcomes (eg, spontaneous abortion, fetal death, stillbirth, congenital anomalies, ectopic pregnancy), as well as any hospitalizations associated with pregnancy complications, unplanned caesarean section, and delivery complications should always be classified as an SAE (as the sponsor considers these to be medically significant), recorded on the eCRF, and expeditiously reported to the sponsor as an SAE following the procedures outlined in Appendix 3, Section 10.3.4.

The subject (or pregnant female partner) will be followed to determine the outcome of the pregnancy. The investigator will collect follow-up information on the subject (or pregnant female partner) and the neonate, and the information will be forwarded to the sponsor.

Any post-study pregnancy-related SAE considered reasonably related to the study treatment by the investigator should be reported to the sponsor as described in Section 8.3.4. While the investigator is not obligated to actively seek this information in former study subjects (or their pregnant female partners), he or she may learn of an SAE through spontaneous reporting.

Pregnancy reporting requirements to BioMarin are shown in Table 8.3.5.1.

Table 8.3.5.1: Pregnancy Reporting to BioMarin

Pregnancy Reporting to BioMarin
• Pregnancy and Pregnancy Outcome information will be entered into the electronic data collection system. • Pregnancy and Pregnancy Outcome information will be reported to BPV via the study specific Pregnancy Report and Pregnancy Outcome paper report forms. The site will complete the forms and submit via facsimile or email without 24 hours of awareness. • Abortion, Stillbirth, Fetal Congenital Anomaly and any other event meeting seriousness criteria must be reported as an SAE via the study electronic data collection system.

Male Subjects with Partners Who Become Pregnant

The investigator will attempt to collect pregnancy information on any male subject's female partner who becomes pregnant while the male subject is in this study. This applies only to male subjects who receive pegvaliase.

After obtaining the necessary signed informed consent from the pregnant female partner directly, the investigator will record pregnancy information on the appropriate form and submit it to the sponsor within 24 hours of learning of the partner's pregnancy. The female partner will also be followed to determine the outcome of the pregnancy. Information on the status of the mother and child will be forwarded to the sponsor. Generally, the follow-up will be no longer than 6 to 8 weeks following the estimated delivery date. Any termination of the pregnancy will be reported regardless of fetal status (presence or absence of anomalies) or indication for the procedure.

8.3.6 Cardiovascular and Death Events

All deaths that occur during the AE reporting period, regardless of attribution, will be recorded on the AE eCRF and expeditiously reported to the Sponsor as an SAE.

When recording a death, the event or condition that caused or contributed to the fatal outcome should be recorded as the single medical concept on the eCRF. If the cause of death is unknown and cannot be ascertained at the time of reporting, record "Unexplained Death" or "Death of Unknown Cause" on the eCRF.

8.3.7 Disease-Related Events and/or Disease-Related Outcomes Not Qualifying as AEs or SAEs

The following disease-related event (DRE) is common in subjects with PKU and can be serious:

- Hyperphenylalaninemia (Medical Dictionary for Regulatory Activities terminology [MedDRA] term: amino acid increased)

Because this event is typically associated with the disease under study, it will not be reported according to the standard process for expedited reporting of SAEs even though the event may meet the definition of a SAE. The elevated blood Phe levels should be recorded in the central laboratory database as per standard practice. These DREs will be monitored on a routine basis.

If either of the following conditions applies, then the event must be recorded and reported as an SAE (instead of a DRE):

- The event is, in the investigator's opinion, of greater intensity, frequency, or duration than expected for the individual subject; OR
- The investigator considers that there is a reasonable possibility that the event was related to study treatment.

8.3.8 Adverse Events of Special Interest

The following adverse events of special interest (AESI) must be reported to BPV within 24 hours of site awareness, irrespective of seriousness, severity, or causality to facilitate real-time monitoring and assessment. All AESI must be reported using the SAE form regardless of seriousness:

- Acute systemic hypersensitivity reaction, anaphylactic, or anaphylactoid reactions (refer to Appendix 5, Section 10.5)
- Angioedema
- Serum sickness
- Severe injection site reaction defined as an injection site reaction that is considered CTCAE Grade ≥ 3
- Hypophenylalaninemia events (defined as blood Phe < 30 µmol/L on 2 consecutive measurements)
- Arthralgia that is either persistent (AE duration > 6 months), severe (CTCAE Grade ≥ 3), or that requires treatment with a steroid (excluding topical)

8.3.8.1 Anaphylaxis (Acute Systemic Hypersensitivity Reaction)

All potential anaphylactic events, however reported, will be reviewed by BioMarin. When an event occurs which an investigator believes is possibly anaphylaxis, in addition to reporting the event to BPV within 24 hours of site awareness, it should be reported to the sponsor's medical monitor within 24 hours of site awareness.

In this protocol the term anaphylaxis is used to denote the important identified risk: acute systemic hypersensitivity reaction (see Section 2.3.1). An acute systemic hypersensitivity reaction (referred to as anaphylaxis in this protocol) is defined as an episode meeting National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network (NIAID/FAAN) criteria according to an internal BioMarin assessment performed by the Internal Data Safety Review Board. The Internal Safety Data Review Board, consisting of a representative from BioMarin clinical sciences, safety, and biostatistics departments, will review all cases of potential anaphylaxis to determine which of these events meet the criteria for an acute systemic hypersensitivity reaction as summarized in Appendix 5, Section 10.5.2.

In the clinical study report, analyses of anaphylaxis will include both the definition used in this protocol of an acute systemic hypersensitivity reaction and the FDA's definition of anaphylaxis, developed by the Division of Pulmonary, Allergy, and Rheumatology Products (DPARP) and used by BioMarin in the Palynziq US prescribing information (see the SAP for further details).

8.3.8.1.1 Individual Stopping Criteria Evaluation for Adverse Events During Pegvaliase Treatment

A subject who has an anaphylaxis episode that is suspected to meet Brown's criteria for severe (Grade 3) and that is, in the judgment of the investigator and/or the sponsor's medical monitor, related to study drug, will be permanently discontinued from study drug. Clinically severe hypersensitivity (Grade 3) per Brown's criteria is defined as significant hypoxia, hypotension or neurologic compromise that is life-threatening or required treatment to prevent a life-threatening event (Brown 2004):

- Cyanosis or $SpO_2 \leq 92\%$
- Hypotension with SBP < 90 mmHg
- Neurologic alteration: confusion, loss of consciousness, collapse, incontinence

In addition, a subject with a recurrent anaphylaxis episode of any severity that is, in the judgment of the investigator and/or the sponsor's medical monitor, related to study drug will be permanently discontinued from treatment.

8.3.8.1.2 Response to Anaphylaxis Episodes

If a subject experiences any symptoms suggestive of an anaphylaxis episode (as taught to the subject and adult observer in the initial and ongoing training on this risk), they will be instructed to inject epinephrine, to seek immediate medical attention, *and* to inform the investigator.

If the investigator suspects that an AE is anaphylaxis as defined by NIAID/FAAN, the subject will be assessed in the clinic and the sponsor's medical monitor must be immediately notified. Laboratory assessments for suspected anaphylaxis events described in the SoA (Section 1.3) under Hypersensitivity Reaction Visit (HRV) and in Table 6.5.1.1 should be performed prior to the next administration of study drug (if applicable) and should include anti-pegvaliase IgE (for optimal results, sampling should be performed 8 to 24 hours after event onset). If the investigator determines it is safe for the subject to resume dosing with study drug following resolution of anaphylaxis at any time during the study (excepting episodes requiring subject discontinuation, as outlined above), the following steps are required:

- At least the first dose administered after resolution of anaphylaxis is to be given at the clinic with equipment for emergency resuscitation (including epinephrine) within easy access.
- The subject must be premedicated with an H1 antagonist, and an H2 antagonist, and an antipyretic (eg, acetaminophen) approximately 2 to 3 hours prior to each dose of study drug for at least 1 week upon resumption of dosing, regardless of the duration of dose interruption.
- As with all doses of pegvaliase, an adult observer must observe the subject during study drug administration and for a minimum of 1 hour following study drug administration.

8.3.8.2 Angioedema

Angioedema is an eruption similar to urticaria but with larger, edematous wheals involving dermal, subcutaneous or submucosal tissues. It is sometimes associated with severe respiratory distress due to edema of the upper airways. Angioedema may present as a symptom (eg, lip, eye, tongue, throat, or facial swelling) of an acute systemic hypersensitivity reaction or anaphylaxis but may also present in isolation.

8.3.8.3 Serum Sickness

Serum sickness is a Type III immune hypersensitivity reaction that occurs when the immune system develops antibodies to a foreign protein. The antibody and foreign protein combine to form an immune complex that can settle in areas of the body such as the joints or the kidney.

Typical presentations include signs and symptoms commonly associated with hypersensitivity or infections: rash, itching, fever and arthralgia (MedDRA terms: serum sickness, serum sickness-like reaction and Type III immune-complex mediated reaction).

8.3.8.4 Severe Injection Site Reaction

A severe injection site reaction will be defined as any injection site reaction adverse event that is reported as CTCAE Grade 3 or above in severity and that codes to the MedDRA high level term (HLT) of Injection site reaction.

8.3.8.5 Hypophenylalaninemia

Blood Phe levels will be regularly monitored during the study. Hypophenylalaninemia is defined in this study as 2 consecutive blood Phe levels < 30 µmol/L. Hypophenylalaninemia will be managed in this study as described in Section 6.5.

8.3.8.6 Arthralgia

Arthralgia will be identified using the MedDRA PT of arthralgia and will include any synonyms of arthralgia such as musculoskeletal pain, pain in the extremity, or neck pain. Any event that meets these criteria and has a duration of > 6 months will be considered a persistent arthralgia. An arthralgia event that is reported as CTCAE Grade 3 or above in severity will be considered a severe event. Arthralgia events that require treatment with a steroid medication will also be considered an AESI.

8.3.9 Medical Device Incidents (Including Malfunctions)

Medical devices (prefilled syringes) are being provided for use in this study. In order to fulfill regulatory reporting obligations, the investigators are responsible for the detection and documentation of events meeting the definitions of device deficiency or malfunction that occur during the study with such devices.

The definition of a Medical Device deficiency can be found in Appendix 6, Section 10.6.

NOTE: Incidents fulfilling the definition of an AE/SAE will also follow the processes outlined in Section 8.3.3 and Appendix 3, Section 10.3.

8.3.9.1 Time Period for Detecting Medical Device Deficiencies

Medical device incidents or malfunctions of the device that result in an incident will be detected, documented, and reported during all periods of the study in which the medical device is used.

If the investigator learns of any device deficiency at any time after a subject has been discharged from the study, and such incident is considered reasonably related to a medical device provided for the study, the investigator will promptly notify the sponsor.

The method of documenting Medical Device deficiencies is provided in Appendix 6, Section 10.6.

8.3.9.2 Follow-up of Medical Device Deficiencies

Follow-up applies to all subjects, including those who discontinue study treatment.

The investigator is responsible for ensuring that follow-up includes any supplemental investigations as indicated to elucidate the nature and/or causality of the deficiency.

New or updated information will be recorded on the originally completed form with all changes signed and dated by the investigator.

8.3.9.3 Prompt Reporting of Device Deficiencies to Sponsor

Device deficiencies must be reported to the sponsor within 24 hours after the investigator determines that the event meets the protocol definition of a medical device deficiency.

The investigator should complete the Device Malfunction eCRF in electronic data capture (EDC). The investigator/designee must complete a Product Complaint form and submit it to the sponsor via fax or email as outlined in the Pharmacy Manual. The sponsor will be the contact for the receipt of device deficiency reports.

8.3.9.4 Regulatory Reporting Requirements for Device Deficiencies

The investigator must promptly report all device deficiencies occurring with any medical device provided for use in the study so the sponsor can fulfill their legal responsibility to notify appropriate regulatory authorities and other entities about certain safety information relating to medical devices being used in clinical studies.

The investigator, or responsible person according to local requirements (eg, the head of the medical institution), will comply with the applicable local regulatory requirements relating to the reporting of device deficiencies to the IRB/IEC.

8.4 Pharmacokinetics

Blood samples will be collected for measurement of plasma concentrations of pegvaliase as specified in the SoA (Section 1.3). For subjects randomized to active (pegvaliase) treatment, Part 1 blood samples for trough PK will be collected pre-dose at Day 1 and every 4 weeks for the first 24 weeks, followed by sampling every 8 weeks thereafter during Part 1. Intensive PK sampling will be performed at Week 73 and at dose escalations to 20, 40, and 60 mg/day.

For intensive PK, samples will be taken at pre-dose and 2, 4, 8, 12, and 24 hours post dose. The 24-hour sample will be taken prior to the next daily dose. For subjects randomized to control (diet-only) treatment, there are no PK assessments in Part 1.

For subjects randomized to active (pegvaliase) treatment, Extension Phase (Part 2) trough PK samples will be collected every 8 weeks. For subjects randomized to control (diet-only) treatment, the Part 2 PK assessment schedule will mirror the Part 1 schedule performed for active subjects, with trough PK samples collected every 4 weeks for the first 24 weeks and then every 8 weeks though the end of the study at Week 153. Intensive PK sampling will be performed Week 145 and for dose escalations to 20, 40, and 60 mg/day.

The PK parameters shown in Table 8.4.1 will be determined if feasible from the plasma concentration-time data for subjects who have sufficient data for PK analysis (eg, at least 3 samples with measurable concentrations during the terminal log-linear phase for estimating the terminal half-life and related parameters; at least 3 samples with measurable concentrations for other derived parameters).

Table 8.4.1: Derived Parameters for Pharmacokinetics Analysis

Parameter	Parameter Description
$AUC_{0-24hr,ss}$	Area under the plasma concentration-time curve from 0 to 24 hours at steady state, calculated using the linear trapezoidal rule
$C_{max,ss}$	Maximum observed plasma concentration within 24 hours post-dose at steady state
$T_{max,ss}$	Time to reach Cmax,ss
$C_{trough,ss}$	Trough observed plasma concentration prior to dosing at steady state
$t_{1/2}$	Terminal half-life, t1/2=ln2/λz where λz is the elimination rate constant
$CL_{ss/F}$	Apparent clearance of drug following subcutaneous injection at steady state
$V_{dz/F}$	Apparent volume of distribution based upon the terminal phase

The timing of sampling may be altered during the course of the study based on newly available data (eg, to obtain data closer to the time of peak plasma concentrations) to ensure appropriate monitoring. Instructions for the collection and handling of biological samples will be provided by the sponsor. The actual date and time (24-hour clock time) of each sample will be recorded.

Plasma samples collected for analyses of pegvaliase concentration may also be used for exploratory safety or efficacy analyses during or after the study.

Genetic analyses will not be performed on these plasma samples.

8.5 Pharmacodynamics

Blood Phe and Tyrosine (Tyr) will be measured by validated assays (see Section 8.1.1 and Section 1.3).

8.6 Biomarkers and Genetic Assessment

8.6.1 PAH Genotyping

PAH genotype testing should be performed where allowed by local health authorities and the EC/IRB.

8.6.2 Blood for Exploratory Analyses

As an exploratory analysis, blood will be collected to evaluate biochemical, molecular, and cellular aspects of PKU and to develop the assays used for these evaluations. The blood samples reserved for exploratory analyses are collected at the time points indicated in the SoA (see Section 1.3).

For each portion of blood samples reserved for protocol-specified analyses, there may be multiple sample aliquots. Once samples have been successfully analyzed during the study per protocol, unused sample portions may be used during the study and afterwards for assay development and other purposes stated in this section.

8.7 Immunogenicity Assessments

Sampling for immunogenicity testing will be performed routinely throughout the study to provide a comprehensive assessment of the immune response against pegvaliase. Antibodies against pegvaliase will be evaluated in serum samples collected from all subjects according to the SoA (Section 1.3). Additionally, serum samples should be collected at the final visit from subjects who discontinued study treatment or were withdrawn from the study.

Routine immunogenicity testing for anti-drug antibodies will be performed using validated semi-quantitative assays used in previous Phase 3 studies. Titer and incidence of positivity for each of the following analytes will be reported: total anti-pegvaliase antibodies (TAb), anti-PAL IgG, anti-PAL IgM, anti-PEG IgM, anti-PEG IgG, and pegvaliase-neutralizing antibodies (NAb). In the event of a hypersensitivity reaction visit (HRV) only, assessment of anti-pegvaliase IgE antibodies will be performed using an ImmunoCAP assay after depletion of IgG and IgM.

8.8 Health Economics/Medical Resource Utilization and Health Economics

Health Economics/Medical Resource Utilization and Health Economics parameters are not evaluated in this study.

9 STATISTICAL CONSIDERATIONS

9.1 Statistical Hypotheses

The study hypothesis is that subjects receiving pegvaliase will show a greater reduction in blood Phe following 72 weeks on study compared to the diet-only control group.

Let μ_a and μ_b denote the mean change from baseline in blood Phe following 72 weeks on study in the pegvaliase and diet-only group, respectively. The null and alternative hypotheses are:

H_0: $\mu_a = \mu_b$

H_1: $\mu_a \neq \mu_b$

The hypotheses will be tested at significance level of 0.05 (two-sided).

9.2 Sample Size Determination

Approximately 54 subjects will be enrolled into the study and randomized to the active (pegvaliase) or control (diet-only) group in 2:1 ratio. Assuming a mean (SD) reduction in Phe following 72 weeks on study of 640 (570) µmol/L in the pegvaliase group and 100 (250) µmol/L in the diet-only group, a total sample size of 54 subjects will provide more than 90% power to detect a treatment difference, based on the two-sample T-test with unequal variance and a significance level of 0.05 (two sided). The assumption of treatment effect is based on clinical data from previous pegvaliase studies and a PKU registry study. See the Statistical Analysis Plan (SAP) for additional details on the sample size calculation.

Approximately 72 subjects will be screened for study participation such that approximately 54 subjects will be enrolled in the study.

Note: Potential subjects who are screened for the purpose of determining eligibility for the study, but do not participate in the study, are not considered enrolled.

9.3 Populations for Analysis

The analysis populations are defined in Table 9.3.1.

Table 9.3.1: Analysis Populations

Population	Description
All Enrolled	All subjects (or their parent/guardians, if applicable) who agree to participate in the clinical study following completion of the informed consent process and determination of study eligibility.
ITT Population	All subjects who are randomized.
Safety Population	All subjects who received study treatment (pegvaliase or diet-only management of PKU).
Per Protocol Population	All subjects in the ITT population who are compliant with the protocol with no major protocol deviations impacting the efficacy analysis. Major protocol deviations impacting efficacy analysis will be defined and documented in the SAP.
PK Population	All subjects with at least one PK measurement

9.4 Statistical Analysis

The SAP will be finalized prior to database lock (DBL) and will include a more technical and detailed description of the statistical analyses described in this section. This section is a summary of the planned statistical analyses of the most important endpoints, including primary and key secondary endpoints.

A clinical pharmacology analysis plan will also be finalized prior to DBL.

Note: If discrepancies exist between the text of the statistical analysis as planned in the protocol, and the SAP, the SAP will prevail.

9.4.1 General Considerations

Descriptive analyses will be performed to gain an understanding of the qualitative and quantitative nature of the data collected and the characteristics of the sample collected. Continuous variables will be reported as mean, standard deviation (SD), median, minimum (min) and maximum (max). Categorical variables will be presented using absolute and relative frequency counts and percentage.

All generation of tables, listings and figures described in the SAP will be performed using SAS® version 9.4 or higher.

Full details on handling missing data and the corresponding analysis methods will be described in detail in the SAP.

9.4.2 Primary Endpoints

The primary efficacy endpoint is the change from baseline in blood Phe following 72 weeks on study. Baseline blood Phe is defined as the mean of 3 pre-treatment measurements:

2 Screening/Run-in measurements to be performed 7 to 10 days apart and the Day 1 pre-dose measurement. The blood Phe measurement following 72 weeks on study is defined as the average of the Week 69 and Week 73 blood Phe measurements.

Change from baseline will be compared between subjects in the active (pegvaliase) arm and the control (diet-only) arm. The primary efficacy endpoint will be evaluated using an Analysis of Covariance (ANCOVA) model, which will include baseline blood Phe as covariate and treatment group as factor, with missing data handled by multiple imputation method. The least square (LS) mean and 95% confidence interval will be calculated for the treatment difference between pegvaliase and diet-only arms following 72 weeks on study. Additional imputation method may be conducted as sensitivity analysis, further details will be provided in the SAP.

9.4.3 Secondary Endpoints

The secondary endpoints are as follows:

- Change from baseline in total dietary protein intake (ie, medical food and/or intact food). Baseline total protein intake is defined as the mean total dietary protein intake calculated from the 3 separate 3-day diet diaries collected during Screening/Run-in. The total dietary protein intake following 72 weeks on study is defined as the mean total dietary protein intake calculated from the diet diaries collected at the Week 69 and Week 73 visits.
- Plasma sample for trough PK

Descriptive summary will be provided. Full details will be provided in the SAP.

9.4.4 Exploratory Endpoints

Refer to the SAP for a discussion of analysis for exploratory endpoints.

9.4.5 Other Safety Analyses

Safety analyses will be performed on the Safety Population. AEs will be coded using the most recent version of the Medical Dictionary for Regulatory Activities (MedDRA). The incidence of treatment-emergent AEs, SAEs, and AESIs will be summarized by MedDRA system organ class, preferred term, relationship to study drug, and severity. Subjects with AEs that result in study drug discontinuation, dosing interruption, or dose level reduction will be tabulated. A by-subject listing of all AEs will be provided.

Clinical laboratory data will be summarized descriptively at baseline and post-baseline visits. Shift tables will be created to summarize the change in CTCAE grade from baseline to worst post-baseline value. Clinically significant laboratory abnormalities reported as AEs will be

summarized. Descriptive statistics for vital signs, physical examination results, ECG test results, and immunogenicity test results will also be provided. Details of statistical methods will be provided in the SAP.

9.5 Interim Analyses

No interim analyses are planned for this study.

9.6 Data Monitoring Committee (DMC)

The independent Data Monitoring Committee (DMC) will act in an advisory capacity to BioMarin to monitor subject safety and the efficacy of pegvaliase in subjects who participate in Study 165-306. The DMC responsibilities may include:

- Review the study protocol, informed consent and assent documents, and plans for data monitoring
- Evaluate the progress of the trial, study data quality, timeliness, subject recruitment, accrual and retention, subjects' risk versus benefit, and other factors that could affect the study outcome
- Consider relevant information that may have an impact on the safety of the subjects or the ethics of the study
- Protect the safety of the study subjects in accordance with the stopping rules as defined in study protocol
- Make recommendations to BioMarin concerning continuation, termination or other modifications of the study based on their observations of the study

Further details are provided in the DMC Charter.

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 Appendix 1: Regulatory, Ethical, and Study Oversight Considerations

10.1.1 Regulatory and Ethical Considerations

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

- Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines
- Principles of Good Clinical Practice as described in the US Code of Federal Regulations and the International Conference on Harmonisation Guidelines
- Applicable laws and regulations

The protocol, protocol amendments, ICF, Investigator Brochure, and other relevant documents (eg, advertisements) as applicable must be submitted, reviewed, and approved by the IRB/IEC before the study is initiated.

Any amendments to the protocol will require IRB/IEC approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study subjects.

The investigator will be responsible for the following:

- Providing written summaries of the status of the study to the IRB/IEC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/IEC
- Notifying the IRB/IEC of SAEs or other significant safety findings as required by IRB/IEC procedures
- Providing oversight of the conduct of the study at the site and adherence to requirements of 21 CFR, ICH guidelines, the IRB/IEC, European regulation 536/2014 for clinical studies (if applicable), and all other applicable local regulations

BioMarin will pay the full costs of the study-related tests, procedures, and treatments set forth in this protocol. In addition, after IRB/IEC approval, BioMarin may reimburse the reasonable cost of travel for study-related visits in accordance with BioMarin's travel and reimbursement policy.

The investigator should contact BioMarin immediately upon notification that a study subject has been injured by the study drug or by procedures performed as part of the study. Any subject who experiences a study-related injury should be instructed by the investigator to

seek immediate medical treatment at a pre-specified medical institution if possible, or at the closest medical treatment facility if necessary. The subject should be given the name of a person to contact to seek further information about, and assistance with, treatment for study-related injuries. BioMarin or the institution may pay for reasonable and necessary medical services to treat the injuries caused by the study drug or study procedures. In some jurisdictions, BioMarin is obligated by law to pay for study-related injuries. If this is the case, BioMarin will comply with the law. BioMarin will not pay for any hospitalizations, tests, or treatments for medical problems of any sort related solely to the study subject's primary disease or any concurrent disease and that are unrelated to the study.

Significant Noncompliance Reporting: Any deviation or series of deviations from the Protocol, ICH GCP, regulatory, and/or legal requirements that is likely to affect to a significant degree the safety or rights of a subject; the stability, quality, and/or control of study drug; or the quality or integrity of the study data shall be reported in writing to BioMarin within one (1) business day upon becoming aware of any suspicion or discovery of Significant Noncompliance.

10.1.2 Statement of Investigator and Financial Disclosure

Prior to beginning the study, the investigator at each site must provide to sponsor or designee a fully executed and signed Statement of Investigator (SOI) form. A US Food and Drug Administration (FDA) Form FDA 1572 serves as an acceptable SOI form. If Form FDA 1572 may not be used in a particular region, the investigator must provide a fully executed SOI on the form provided by the sponsor. All investigators and sub-investigators must be listed on Form FDA 1572 or its equivalent SOI. Financial Disclosure Forms must be completed with sufficient, accurate financial information as requested for all investigators and sub-investigators listed on the Form FDA 1572 or SOI who will be directly involved in the treatment or evaluation of subjects in this study. The Financial Disclosure Forms allow the sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities. Investigators must submit an updated Financial Disclosure Form to BioMarin should their response to any questions within the form or disclosure status change during the period of the study and for one year after study completion.

In accordance with FDA Form 1572 and/or principles of ICH GCP, the investigator will ensure that:

- He or 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 or she will personally conduct or supervise the study.
- He or 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 CFR Part 50 and/or ICH Sections 2.9 and 4.8 are met. As well, he or she will ensure that IRB/IEC review and approval in 21 CFR Part 56 and/or ICH Section 2.6 are met.
- He or she will report to the sponsor adverse experiences that occur in the course of the investigation in accordance with 21 CFR 312.64 and/or ICH Section 4.11.
- He or she has read and understands the information in the IB, including potential risks and side effects of the drug.
- His or her staff and all persons who assist in the conduct of the study are informed about their obligations in meeting the above commitments
- Adequate and accurate records in accordance with 21 CFR 312.62 and/or ICH Section 4.9 are kept, and those records are available for inspection in accordance with 21 CFR 312.68 and/or ICH Section 4.9.7.
- The IRB/EC/REB complies with the requirements of 21 CFR Part 56, ICH Section 3.0, and other applicable regulations, and conducts initial and ongoing reviews and approvals of the study. He or 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/REB. Additionally, he or she will not make any changes in the research without IRB/EC/REB approval, except where necessary to eliminate apparent immediate hazards to human subjects.
- He or she agrees to comply with all other requirements regarding the obligations of clinical Investigators and all other pertinent requirements in 21 CFR Part 312 and/or ICH E6 R2.

10.1.3 Informed Consent Process

The investigator or his/her representative will explain the nature of the study to the subject or his/her legally authorized representative and answer all questions regarding the study.

Subjects must be informed that their participation is voluntary. Subjects or their legally authorized representative (as determined by the laws of the jurisdiction in which the study is being conducted) will be required to sign a statement of informed consent that meets the regulatory requirements of the country or region where the subject consents, such as 21 CFR 50 (US FDA), ICH guidelines, privacy requirements including but not limited to the Health Insurance Portability and Accountability Act (HIPAA) requirements and the General Data Protection Regulation (where applicable), and the IRB/IEC or study center. If there is no applicable law addressing the issue of who may be a legally authorized representative, a legally authorized representative will be an individual recognized by institutional policy as

acceptable for providing consent on behalf of the prospective subject to the subject's participation in the procedure(s) involved in the research.

The medical record must include a statement that written informed consent was obtained before any study specific procedures were initiated and the date the written consent was obtained. The authorized person obtaining the informed consent must also sign the ICF.

Subjects must be re-consented to the most current version of the ICF(s) during their participation in the study. Subjects who reach the age of majority in their country while the study is ongoing will be asked to provide their own written consent again upon reaching the legal age of majority.

The ICF will contain a separate section that addresses the use of remaining samples for optional additional research. The investigator or authorized designee will explain to each subject the objectives of the additional research. Subjects will be told that they are free to refuse to participate and may withdraw their consent at any time and for any reason during the storage period. A separate signature will be required to document a subject's agreement to allow any remaining samples to be used for additional research.

A copy of the ICF(s) must be provided to the subject or the subject's legally authorized representative.

A subject who is rescreened is not required to sign another ICF if the rescreening occurs during the Screening/Run-in period.

10.1.4 Data Protection

Measures will be taken to ensure the privacy of subject data, including the use of subject numbers on eCRFs. A list linking subject identification numbers with subject names and other personal information will be kept in a secure place, separate from the subjects' medical records. BioMarin will follow all applicable regulations and guidelines in the relevant locality, country, and/or region to protect the privacy of subject data.

Subjects will be assigned a unique identifier by the sponsor. Any subject records or datasets that are transferred to the sponsor will contain the identifier only; subject names or any information which would make the subject identifiable will not be transferred.

The subject must be informed that his/her personal study-related data will be used by the sponsor in accordance with local data protection law. The level of disclosure must also be explained to the subject, who will be required to give consent or be given notice of the legal bases for their data to be used as described in the informed consent.

The subject must be informed that his/her medical records may be examined by authorized personnel appointed by the sponsor, by appropriate IRB/IEC members, and by inspectors from regulatory authorities.

10.1.5 Committee Structures

The following advisory and assurance activities will be implemented to support the study: monitoring committees, clinical trial disclosure, and study monitoring.

10.1.5.1 Data Monitoring Committee (DMC)

An external independent Data Monitoring Committee (DMC) will be responsible for monitoring subject safety and efficacy of pegvaliase. The DMC consisting of five members, who are each independent of the conduct of studies in the BioMarin BMN 165 (pegvaliase) development program, and are experts in the fields of PKU, clinical immunology, neuropsychology, pharmacovigilance, and biostatistics. DMC responsibilities are described in Section 9.6.

10.1.5.2 Internal Safety Data Review Board

The Internal Safety Data Review Board, consisting of a representative from BioMarin clinical sciences, safety, and biostatistics, will review all cases of potential anaphylaxis to determine which of these events meet the criteria for an acute systemic hypersensitivity reaction as summarized in Appendix 5, Section 10.5.2.

10.1.6 Dissemination of Clinical Study Data

The posting of study information and study results will comply with applicable national regulatory requirements and BioMarin's data sharing policy available at https://www.biomarin.com/data-request-form.

10.1.7 Data Quality Assurance

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

Sites will enter study data into eCRFs in the study EDC system. Data Quality Control will be performed by BioMarin Clinical Data Management or a designee through implementation of quality control checks specified in the study data management plan and edit check specifications.

Study monitors will perform ongoing source data verification to confirm that data entered into the eCRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of subjects are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements. Qualified individuals designated by BioMarin will monitor all aspects of the study according to 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 subjects, and, if requested, agrees to assist the monitors. The investigator and staff are responsible for being present or available for consultation during routinely scheduled site visits conducted by BioMarin or its designees.

The investigator must retain all study records required by BioMarin and by the applicable regulations in a secure and safe facility. The investigator must notify BioMarin 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, that is, 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, subject 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) at least 2 years have elapsed since the formal discontinuation of clinical development of the IP. The investigator/institution should retain subject identifiers for at least 15 years after the completion or discontinuation of the study. Subject 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 a BioMarin agreement. BioMarin must be notified and will assist with retention should the investigator/institution be unable to continue maintenance of subject files for the full 15 years. It is the responsibility of BioMarin to inform the investigator/institution as to when these documents no longer need to be retained.

Representatives of BioMarin or designees may conduct an audit of a site at any time before, 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 inspection of the study. If informed of such an inspection, the investigator should notify BioMarin immediately. The investigator will ensure that the auditors/inspectors have access to the clinical supplies, site facilities, original source documentation, and all study files.

10.1.8 Source Documents

Source documents provide evidence for the existence of the subject and substantiate the integrity of the data collected. Source documents are filed at the investigator's site.

Data entered in the eCRF that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The investigator may need to request previous medical records or transfer records, depending on the study. Also, current medical records must be available.

The definition of what constitutes source data can be found in the Monitoring Plan.

10.1.9 Study and Site Start and Closure

First Act of Recruitment

The study start date is the date on which the clinical study will be open for recruitment of subjects.

The first act of recruitment is the first site open and will be the study start date.

Study/Site Termination

The sponsor reserves the right to close the site or terminate the study at any time for any reason at the sole discretion of the sponsor. Sites will be closed upon study completion. A site is considered closed when all required documents and unused study supplies have been collected and a site closure visit has been performed.

Reasons for the early closure of a site by the sponsor may include but are not limited to:

- Failure of the investigator to comply with the protocol, the requirements of the IRB/IEC or local health authorities, the sponsor's procedures, or GCP guidelines
- Inadequate recruitment of subjects by the investigator
- Discontinuation of further study treatment development

If the study is prematurely terminated or suspended, the sponsor shall promptly inform the investigators, the IECs/IRBs, the regulatory authorities, and any contract research organization(s) used in the study of the reason for termination or suspension, as specified by the applicable regulatory requirements. The investigator shall promptly inform the subject(s) and should assure appropriate subject therapy and/or follow-up.

10.1.10 Publication Policy

The results of this study may be published or presented at scientific meetings. If this is foreseen, the investigators agree to submit all manuscripts or abstracts to the sponsor at least forty-five (45) days before submission or as agreed in the Clinical Trial Agreement between BioMarin and the investigator/institution. This allows the sponsor to protect proprietary information and to provide comments.

The sponsor will comply with the requirements for publication of study results. In accordance with standard editorial and ethical practice, the sponsor will generally support publication of multicenter studies only in their entirety and not as individual site data. In this case, a coordinating investigator will be designated by mutual agreement.

BioMarin recognizes the importance of communicating medical study data and therefore encourages the publication of these data 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 may be described in the Clinical Trial Agreement between BioMarin and the investigator/institution. Consideration for authorship of all publications will be based on mutual agreement and compliance with the Uniform Requirements for Manuscripts Submitted to Biomedical Journals (“Uniform Requirements”) of the International Committee of Medical Journal Editors (ICMJE) (http://www.icmje.org/about-icmje/faqs/icmje-recommendations/) and good publication practices (GPP).

10.2 Appendix 2: Clinical Laboratory Tests

The tests detailed in Table 10.2.1 will be performed by the central laboratory.

Protocol-specific requirements for inclusion or exclusion of subjects are detailed in Section 5 of the protocol.

Additional tests may be performed at any time during the study as determined necessary by the investigator or required by local regulations.

Table 10.2.1: Clinical Laboratory Tests

Laboratory Test	Parameters		
Chemistry	Albumin Alkaline Phosphatase ALT (SGPT) AST (SGOT) Bicarbonate Direct bilirubin Total bilirubin BUN	Calcium Total cholesterol CO_2 Creatinine Creatine phosphokinase Glucose (fasting) GGT	LDH Phosphorus Potassium Total protein Sodium Uric acid
Hematology	Hemoglobin Hematocrit	WBC count RBC count	Platelet count Differential cell count
Urinalysis	Appearance Color pH Specific gravity Ketone Protein	Glucose Bilirubin Nitrite Urobilinogen Hemoglobin	Urinary albumin/creatinine ratio [a] Urinary microscopy Urinary N-methyl histamine
Pregnancy testing	Refer to Section 8.2.5		
Complement panel	C3 C4		
Other tests conducted during the study (refer to the SoA for timing)	Phenylalanine Tyrosine hs-CRP Serum tryptase level Serum cortisol [b] Erythrocyte sedimentation rate [c]		

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; C3, C4, complement components 3, 4; CO2, carbon dioxide; ESR, Erythrocyte sedimentation rate; GGT, gamma-glutamyltransferase; hs-CRP, high sensitivity C-reactive protein; LDH, lactate dehydrogenase; RBC, red blood cell; SGOT, serum glutamic-oxaloacetic transaminase; SGPT, serum glutamic-pyruvic transaminase; WBC, white blood cell.

[a] It is recommended that urine samples be obtained as a first or second morning void. Subjects with a confirmed urine/albumin creatinine ratio of ≥ 100 mg/g should be referred to a nephrologist for consultation if results were within normal range at Baseline. Subjects who had elevated results at Baseline followed by a confirmed subsequent increase of 100-200 mg/g from Baseline should also be referred to a nephrologist for consultation.

[b] Serum cortisol samples should be taken before study drug administration in the morning before 10:00 am. A home healthcare nurse may collect samples for subject convenience. If 2 results are low and abnormal, the subject will be asked to perform additional sampling for plasma ACTH and a low-dose conventional ACTH stimulation test or the ACTH test method based on the clinical practice at the site.

[c] ESR will be performed at a local laboratory.

Investigators must document their review of each laboratory safety report.

10.3 Appendix 3: Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting

10.3.1 Definition of an Adverse Event

AE Definition
• An AE is any untoward medical occurrence in a patient or clinical study subject, temporally associated with the use of study treatment, whether or not considered related to the study treatment. • NOTE: An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of study treatment.

Events Meeting the AE Definition
• Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (eg, ECG, radiological scans, vital signs measurements), including those that worsen from baseline, considered clinically significant in the medical and scientific judgment of the investigator (ie, not related to progression of underlying disease). • Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition. • New conditions detected or diagnosed after study treatment administration • Signs, symptoms, or the clinical sequelae of a suspected drug-drug interaction. • Signs, symptoms, or the clinical sequelae of a suspected overdose of either study treatment or a concomitant medication. Overdose per se will not be reported as an AE/SAE unless it is an intentional overdose taken with possible suicidal/self-harming intent. Such overdoses should be reported regardless of sequelae. • "Lack of efficacy" or "failure of expected pharmacological action" per se will not be reported as an AE or SAE. Such instances will be captured in the efficacy assessments. However, the signs, symptoms, and/or clinical sequelae resulting from lack of efficacy will be reported as AE or SAE if they fulfill the definition of an AE or SAE.

Events Not Meeting the AE Definition
• Any clinically significant abnormal laboratory findings or other abnormal safety assessments which are associated with the underlying disease, unless judged by the investigator to be more severe than expected for the subject's condition. • The disease/disorder being studied or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the subject's condition. • Medical or surgical procedure (eg, endoscopy, appendectomy): the condition that leads to the procedure is the AE. • Situations in which an untoward medical occurrence did not occur (social and/or convenience admission to a hospital).

- Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.
- Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline
- Planned hospitalization for childbirth (eg, scheduled Caesarean section)

10.3.2 Definition of an SAE

If an event is not an AE per the definition above, then it cannot be an SAE even if serious conditions are met (eg, hospitalization for signs/symptoms of the disease under study, death due to progression of disease).

A SAE is defined as any untoward medical occurrence that, at any dose:
a. Results in death
b. Is life-threatening The term "life-threatening" in the definition of "serious" refers to an event in which the subject was at risk of death at the time of the event. It does not refer to an event that hypothetically might have caused death if it were more severe.
c. Requires inpatient hospitalization or prolongation of existing hospitalization In general, hospitalization signifies that the subject has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious.
d. Results in persistent disability/incapacity The term disability means a substantial disruption of a person's ability to conduct normal life functions. This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (eg, sprained ankle) which may interfere with or prevent everyday life functions but do not constitute a substantial disruption.
e. Is a congenital anomaly/birth defect in the offspring of a study subject
f. Other situations/Important Medical Events: Medical or scientific judgment should be exercised in deciding whether SAE reporting is appropriate in other situations such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require medical or surgical treatment to prevent one of the other outcomes listed in the above definition. These events should usually be considered serious. Examples of such events include invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

10.3.3 Recording and Follow-Up of AEs and/or SAEs

AE and SAE Recording

- When an AE/SAE occurs, it is the responsibility of the investigator to review all documentation (eg, hospital progress notes, laboratory reports, and diagnostics reports) related to the event.
- The investigator or qualified designee will then record all relevant AE/SAE information in the eCRF.
- It is **not** acceptable for the investigator to send photocopies of the subject's medical records to BioMarin in lieu of completion of the AE/SAE eCRF page.
- There may be instances when copies of medical records for certain cases are requested by BioMarin. In these cases, all subject identifiers, with the exception of the subject number, will be redacted on the copies of the medical records before submission to BioMarin.
- The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. Whenever possible, the diagnosis (not the individual signs/symptoms) will be documented as the AE/SAE.

Assessment of Intensity

The investigator will make an assessment of intensity for each AE and SAE reported during the study and assign it to 1 of the following categories (as defined in Common Terminology Criteria for Adverse Events [CTCAE] version 5.0). The investigator should only record the single highest grade of intensity for each AE and SAE in the eCRF. Adverse events that do not have a corresponding CTCAE term will be assessed according to the general guidelines for grading:

- Grade 1 (Mild): asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated
- Grade 2: (Moderate): minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL) (eg, preparing meals, shopping for groceries or clothes, using the telephone, managing money)
- Grade 3 (Severe): medically significant but not immediately life-threatening: hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL (eg, bathing, dressing/undressing, feeding oneself, using the toilet, taking medications, not bedridden)
- Grade 4: Life threatening consequences; urgent intervention indicated
- Grade 5: Death related to AE

An event is defined as "serious" when it meets at least 1 of the predefined outcomes as described in the definition of an SAE, NOT when it is rated as severe.

Assessment of Causality

- The investigator is obligated to assess the relationship between study treatment and each occurrence of each AE/SAE.
- An assessment of "related" means that there are facts, evidence, and/or arguments to suggest a causal relationship such that a relationship cannot be ruled out.
- The investigator will use clinical judgment to determine the relationship.

- Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to study treatment administration will be considered and investigated.
- For each AE/SAE, the investigator must document in the medical notes that he/she has reviewed the AE/SAE and has provided an assessment of causality.
- There may be situations in which an SAE has occurred, and the investigator has minimal information to include in the initial report to BioMarin. However, it is very important that the investigator always make an assessment of causality for every event before the initial transmission of the SAE data to BioMarin.
- The investigator may change his/her opinion of causality in light of follow-up information and send a SAE follow-up report with the updated causality assessment.

Follow-up of AEs and SAEs

- The investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by BioMarin to elucidate the nature and/or causality of the AE or SAE as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.
- If a subject dies during participation in the study or during a recognized follow-up period, the investigator will provide a copy of any post-mortem findings including histopathology.
- New or updated information will be recorded in the originally completed eCRF.
- The investigator will submit any updated SAE data to the sponsor within 24 hours of receipt of the information.

10.3.4 Reporting of SAEs and Events of Special Interest

SAE/AESI Reporting to BioMarin Pharmacovigilance (BPV) via an Electronic Data Collection Tool

- The primary mechanism for reporting an SAE/AESI to BPV will be the electronic data collection tool. AESI reporting will follow the SAE reporting requirements.
- If the electronic system is unavailable, then the site will use the paper SAE data collection tool (see next section) in order to report the event within 24 hours.
- The site will enter the SAE/AESI data into the electronic system as soon as it becomes available.
- After the study is completed at a given site, the electronic data collection tool will be taken off-line to prevent the entry of new data or changes to existing data.
- If a site receives a report of a new SAE/AESI from a study subject or receives updated data on a previously reported SAE/AESI after the electronic data collection tool has been taken off-line, then the site can report this information on a paper SAE form (see next section) or to BPV by telephone.

SAE/AESI Reporting to BPV via Paper CRF
• Facsimile transmission or email of the paper CRF (pregnancy form, pregnancy outcome, or SAE form is EDC unavailable) is the preferred method to transmit this information to BPV. • In rare circumstances and in the absence of facsimile equipment, notification by telephone is acceptable with a copy of the paper CRF sent by overnight mail or courier service. • Initial notification via telephone does not replace the need for the investigator to complete and sign the CRF pages within the designated reporting time frames.

Contact information for BioMarin Pharmacovigilance is as follows:

BioMarin Pharmaceutical Inc.
Address 105 Digital Drive
Novato, CA 94949
Phone: PI
Fax:
E-mail: drugsafety@bmrn.com

The Investigator is encouraged to discuss with the medical monitor any AEs for which the issue of seriousness is unclear or questioned. Contact information for the medical monitor is provided on the signature page at the front of this protocol.

Name: PI RN, MS
Address: 105 Digital Drive
Novato, CA 94949 USA
Phone: PI
Fax:
E-mail: PI

10.4 Appendix 4: Contraceptive and Barrier Guidance

Definitions:

Woman of Childbearing Potential (WOCBP)

A woman is considered fertile following menarche and until becoming post-menopausal unless permanently sterile (see below).

If fertility is unclear (eg, amenorrhea in adolescents or athletes) and a menstrual cycle cannot be confirmed before first dose of study treatment, additional evaluation should be considered.

Women in the following categories are not considered WOCBP:

- Premenarchal
- Premenopausal female with 1 of the following:
    - Documented hysterectomy
    - Documented bilateral salpingectomy
    - Documented bilateral oophorectomy

  For individuals with permanent infertility due to a medical cause other than the above (eg, mullerian agenesis, androgen insensitivity), investigator discretion should be applied in determining study eligibility.

  Note: Documentation can come from the site personnel's review of the subject's medical records, medical examination, or medical history interview.
- Postmenopausal female
    - A postmenopausal state is defined as no menses for 12 months without an alternative medical cause.
        - A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a postmenopausal state in women not using hormonal contraception or hormonal replacement therapy (HRT). However, in the absence of 12 months of amenorrhea, confirmation with more than one FSH measurement is required.
    - Females on HRT whose menopausal status is in doubt will be required to use one of the non-estrogen hormonal highly effective contraception methods if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of postmenopausal status before study enrollment.

Contraception Guidance:

If sexually active, must be willing to use two acceptable methods of contraception while participating in the study and 4 weeks after the last dose of pegvaliase.

10.5 Appendix 5: Anaphylaxis Criteria

For the purposes of this study, the term anaphylaxis refers to the important identified risk of acute systemic hypersensitivity reaction. An acute systemic hypersensitivity reaction is one that is consistent with the NIAID/FAAN definition as assessed by BioMarin using the criteria listed below in Appendix 5, Section 10.5.1 and Appendix 5, Section 10.5.2.

10.5.1 Sampson's Anaphylaxis Criteria

According to the National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network (NIAID/FAAN) Second Symposium on the definition and management of anaphylaxis, anaphylaxis is highly likely when any one of the following 3 criteria are fulfilled:

1. Acute onset of an illness (minutes to several hours) with involvement of the skin, mucosal tissue, or both (eg, generalized hives, pruritus or flushing, swollen lips-tongue-uvula)

   AND AT LEAST ONE OF THE FOLLOWING:

   a. Respiratory compromise (eg, dyspnea, wheeze-bronchospasm, stridor, reduced peak expiratory flow [PEF], hypoxemia)

   b. Reduced blood pressure (BP) or associated symptoms of end-organ dysfunction (eg, hypotonia [collapse], syncope, incontinence)

2. Two or more of the following that occur rapidly after exposure *to a likely allergen for that patient* (minutes to several hours):

   a. Involvement of the skin-mucosal tissue (eg, generalized hives, itch-flush, swollen lips-tongue-uvula)

   b. Respiratory compromise (eg, dyspnea, wheeze-bronchospasm, stridor, reduced PEF, hypoxemia)

   c. Reduced BP or associated symptoms (eg, hypotonia [collapse], syncope, incontinence)

   d. Persistent gastrointestinal symptoms (eg, crampy abdominal pain, vomiting)

3. Reduced BP after exposure to *known allergen for that patient* (minutes to several hours):

a. Infants and children: low systolic blood pressure (age specific) or greater than 30% decrease is systolic BP

b. Adults: systolic BP of less than 90 mmHg or greater than 30% decrease from that person's baseline.

Source: Sampson 2006.

10.5.2 Acute Systemic Hypersensitivity Reaction

An acute systemic hypersensitivity reaction is a Type III acute systemic allergic reaction. An acute systemic hypersensitivity reaction has a similar clinical presentation to an anaphylactic reaction, but only meets the definition if the italicized criteria below are met in addition to the criteria outlined in Sampson's criteria (see Appendix 5, Section 10.5.1, Sampson's Criteria):

Sampson Criteria Number	Sampson Criteria Wording With Additional Criteria Necessary to Meet Acute Systemic Hypersensitivity Reaction Criteria Shown in Italics
1	Acute onset of an illness (minutes to several hours) with involvement of the skin, mucosal tissue, or both (eg, generalized hives, pruritus or flushing, swollen lips-tongue-uvula). *Must be generalized skin reaction to meet criteria*.
1a	Respiratory compromise (eg, dyspnea, wheeze-bronchospasm, stridor, reduced PEF, hypoxemia). *Respiratory compromise is demonstrated by oxygen desaturation below 88% on room air.*
1b	Reduced BP or associated symptoms of end-organ dysfunction (eg, hypotonia [collapse], syncope, incontinence). *Systolic BP less than 90 mmHg (adults) or 70 mmHg (children) or greater than 30% decrease from baseline*.
2d	Persistent gastrointestinal symptoms (eg, crampy abdominal pain, vomiting). *Gastrointestinal symptoms are persistent with multiple episodes that continue for several hours or more*.

10.5.3 Anaphylaxis Analysis

During the initial BLA for Palynziq in the US, the FDA proposed that anaphylaxis should be defined for labelling purposes using the methodology developed by FDA's Division of Pulmonary, Allergy, and Rheumatology Products (DPARP). As a result, in the study analyses for 165-306 anaphylaxis, including derivation of the incidence and event rates, data will be presented using both BioMarin's definition of an acute systemic hypersensitivity reaction described above and the FDA's assessment methodology, ie, a case will be counted as an anaphylaxis event if it meets any of the following criteria:

- Clinical manifestations consistent with NIAID/FAAN criterion #1 (skin involvement + respiratory or cardiovascular compromise); or

- Verbatim reports of anaphylaxis/anaphylactic reaction (or synonyms), even if NIAID/FAAN criteria were not met; or
- Cases involving subject injection of epinephrine alone, even if the subject did not experience signs/symptoms necessary to meet NIAID/FAAN criteria.

Further details are provided in the SAP.

10.6 Appendix 6: Medical Device Adverse Events, Adverse Device Effects (ADEs), Serious Adverse Events (SAEs), and Device Deficiencies: Definition and Procedures for Recording, Evaluating, Follow-Up, and Reporting

Medical Device Adverse Event reporting applies only to US sites. The definitions and procedures detailed in this appendix are in accordance with ISO 14155.

Both the investigator and the sponsor will comply with all local medical device reporting requirements.

The detection and documentation procedures described in this protocol apply to all sponsor medical devices provided for use in the study. For this study, prefilled syringes are provided (see Section 6.1.1).

10.6.1 Definition of AE and ADE

AE and ADE Definition

- An AE is defined as any untoward medical occurrence, unintended disease or injury, or untoward clinical signs (including abnormal laboratory finding) in study subjects, users, or other persons, whether or not related to the investigational medical device. This definition includes events related to the investigational medical device or comparator and events related to the procedures involved except for events in users or other persons, which only include events related to investigational devices.
- An adverse device effect (ADE) is defined as an adverse event related to the use of an investigational medical device. This definition includes any adverse events resulting from insufficient or inadequate instructions for use, deployment, implantation, installation, or operation, or any malfunction of the investigational medical device as well as any event resulting from use error or from intentional misuse of the investigational medical device.

10.6.2 Definition of SAE, SADE, and USADE

If an event is not an AE per definition above, then it cannot be an SAE even if serious conditions are met (eg, hospitalization for signs/symptoms of the disease under study, death due to disease progression).

An SAE is an AE that:

- Led to death
- Led to serious deterioration in the health of the subject resulting in either:
  1. A life-threatening illness or injury. The term 'life-threatening' in the definition of 'serious' refers to an event in which the subject was at risk of death at the time of the event. It does not refer to an event which hypothetically might have caused death if it were more severe.
  2. A permanent impairment of a body structure or a body function
  3. Inpatient or prolonged hospitalization, planned hospitalization for a pre-existing condition, or a procedure required by the protocol, without serious deterioration in health, is not considered an SAE.
  4. Medical or surgical intervention to prevent life-threatening illness or injury or permanent impairment to a body structure or a body function
- Led to fetal distress, fetal death, or a congenital abnormality or birth defect

SADE definition:

- A SADE is defined as an adverse device effect that has resulted in any of the consequences characteristic of a serious adverse event.

USADE definition

- A USADE is a serious adverse device effect which by its nature, incidence, severity, or outcome has not been identified in the current version of the risk analysis report.

10.6.3 Definition of Device Deficiency

Device deficiency definition

- A device deficiency is an inadequacy of a medical device with respect to its identity, quality, durability, reliability, safety, or performance. Device deficiencies include malfunctions, use errors, and inadequate labeling.

10.6.4 Recording and Follow-Up of AE and/or SAE and Device Deficiencies

AE, SAE, and Device Deficiency Recording

- When an AE/SAE/device deficiency occurs, it is the responsibility of the investigator to review all documentation (e.g., hospital progress notes, laboratory reports, and diagnostics reports) related to the event.
- The investigator will then record all relevant AE/SAE/device deficiency information in the subject's medical records, in accordance with the investigator's normal clinical practice and on the appropriate form of the eCRF.
- All ADEs will be entered into the AE eCRF.
- SADEs will be entered into the AE eCRF and submitted to BPV. The site will also complete and submit the study specific paper device report form within 24 hours of site awareness.
- It is not acceptable for the investigator to send photocopies of the subject's medical records to BPV in lieu of completion of the /AE/SAE/device deficiency eCRF page.
- There may be instances when copies of medical records for certain cases are requested by BPV. In this case, all subject identifiers, with the exception of the subject number, will be redacted on the copies of the medical records before submission to BPV.
- The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. Whenever possible, the diagnosis (not the individual signs/symptoms) will be documented as the AE/SAE.
- For device deficiencies, it is very important that the investigator describes any corrective or remedial actions taken to prevent recurrence of the incident.
- A remedial action is any action other than routine maintenance or servicing of a medical device where such action is necessary to prevent recurrence of a device deficiency. This includes any amendment to the device design to prevent recurrence.

Assessment of Intensity

- The investigator will make an assessment of intensity for each AE/SAE/device deficiency reported during the study and assign it to one of the following categories:
  - Mild: An event that is easily tolerated by the subject, causing minimal discomfort and not interfering with everyday activities.
  - Moderate: An event that causes sufficient discomfort and interferes with normal everyday activities.
  - Severe: An event that prevents normal everyday activities. An AE that is assessed as severe should not be confused with a SAE. Severe is a category utilized for rating the intensity of an event; and both AEs and SAEs can be assessed as severe.
- An event is defined as 'serious' when it meets at least 1 of the predefined outcomes as described in the definition of an SAE, NOT when it is rated as severe.

Assessment of Causality

- The investigator is obligated to assess the relationship between study treatment and each occurrence of each AE/SAE/device deficiency.
- A "reasonable possibility" of a relationship conveys that there are facts, evidence, and/or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out.
- The investigator will use clinical judgment to determine the relationship.
- Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to study treatment administration will be considered and investigated.
- The investigator will also consult the IB in his/her assessment.
- For each AE/SAE/device deficiency, the investigator must document in the medical notes that he/she has reviewed the AE/SAE/device deficiency and has provided an assessment of causality.
- There may be situations in which an SAE has occurred and the investigator has minimal information to include in the initial report to BPV. However, it is very important that the investigator always make an assessment of causality for every event before the initial transmission of the SAE data to BPV.
- The investigator may change his/her opinion of causality in light of follow-up information and send a SAE follow-up report with the updated causality assessment.
- The causality assessment is one of the criteria used when determining regulatory reporting requirements.

Follow-up of AE/SAE/device deficiency

- The investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by BPV to elucidate the nature and/or causality of the AE/SAE/device deficiency as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.
- If a subject dies during participation in the study or during a recognized follow-up period, the investigator will provide BPV with a copy of any post mortem findings including histopathology.
- New or updated information will be recorded in the originally completed eCRF.
- The investigator will submit any updated SAE data to BPV within 24 hours of receipt of the information.

10.6.5 Reporting of SAEs

SAE Reporting to BPV via an Electronic Data Collection Tool

- The primary mechanism for reporting an SAE to BPV will be the electronic data collection tool.
- If the electronic system is unavailable, then the site will use the paper SAE data collection tool (see next section) in order to report the event within 24 hours.
- The site will enter the SAE data into the electronic system as soon as it becomes available.
- After the study is completed at a given site, the electronic data collection tool will be taken off-line to prevent the entry of new data or changes to existing data.
- If a site receives a report of a new SAE from a study subject or receives updated data on a previously reported SAE after the electronic data collection tool has been taken off-line, then the site can report this information on a paper SAE form (see next section) or to BPV by telephone.
- Contacts for SAE reporting can be found in Appendix 3, Section 10.3.4.

SAE Reporting to BPV via Paper CRF

- Facsimile transmission of the SAE paper CRF is the preferred method to transmit this information to BPV.
- In rare circumstances and in the absence of facsimile equipment, notification by telephone is acceptable with a copy of the SAE data collection tool sent by overnight mail or courier service.
- Initial notification via telephone does not replace the need for the investigator to complete and sign the SAE CRF pages within the designated reporting time frames.
- Contacts for SAE reporting can be found in Appendix 3, Section 10.3.4.

10.6.6 Reporting of SADEs

SAE Reporting to BPV

NOTE: There are additional reporting obligations for medical device incidents that are potentially related to SAEs that must fulfil the legal responsibility to notify appropriate regulatory authorities and other entities about certain safety information relating to medical devices being used in clinical studies.

- Any device deficiency that is associated with an SAE must be reported to the sponsor within 24 hours after the investigator determines that the event meets the definition of a device deficiency.
- The sponsor shall review all device deficiencies and determine and document in writing whether they could have led to an SAE. These shall be reported to the regulatory authorities and IRBs/IECs as required by national regulations.
- Contacts for SAE reporting can be found in Appendix 3, Section 10.3.4.

10.7 Appendix 7: Abbreviations

Abbreviation	Definition
ACMG	American College of Medical Genetics and Genomics
ADHD	Attention Deficit Hyperactivity Disorder
ADHD-RS	Attention Deficit Hyperactivity Disorder Rating Scale
ADL	activities of daily living
AE	adverse event
AESI	adverse events of special interest
ALT	alanine aminotransferase
ANCOVA	Analysis of Covariance
AST	aspartate aminotransferase
AUC	area under the concentration-time curve
BH4	tetrahydrobiopterin
BLA	Biologics License Application
BPV	BioMarin Pharmacovigilance
BRIEF	Behavior Rating Inventory of Executive Function
C3	complement component 3
C4	complement component 4
CIOMS	Council for International Organizations of Medical Sciences
C_{max}	maximum observed plasma concentration
CONSORT	Consolidated Standards of Reporting Trials
CRF	case report form
CRP	C-reactive protein
CTCAE	Common Terminology Criteria for Adverse Events
DBL	database lock
DFRF	Directed Follow-up Report Form
DMC	Data Monitoring Committee
DPARP	FDA Division of Pulmonary, Allergy, and Rheumatology Products
DRE	disease-related event
DRI	Dietary Reference Intake
DSM	Diagnostic and Statistical Manual of Mental Disorders
ECG	electrocardiogram
EC/IRB	Ethics Committee/Institutional Review Board
eCRF	electronic Case Report Form
EDC	electronic data capture
ET	Early Termination

Abbreviation	Definition
EU	European Union
FDA	Food and Drug Administration
FSH	follicle stimulating hormone
GCP	good clinical practice
GPP	good publication practice
HAE	hypersensitivity adverse event
HIPAA	Health Insurance Portability and Accountability Act
HPA	hyperphenylalaninemia
HRT	hormonal replacement therapy
HRV	hypersensitivity reaction visit
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICMJE	International Committee of Medical Journal Editors
IgE	immunoglobulin E
IgG	immunoglobulin G
IgM	immunoglobulin M
IMP	investigational medical products
IND	investigational new drug
IP	investigational product
IQ	intelligence quotient
ISR	injection site reaction
I/T/M	Induction/Titration/Maintenance (dosing regimen)
LS	least square
MedDRA	Medical Dictionary for Regulatory Activities
MNT	medical nutritional therapy
NAb	neutralizing antibody
NCI-CTCAE	National Cancer Institute – Common Terminology Criteria for Adverse Events
NIAID/FAAN	National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network
NIMP	non-investigational medical products
NPKUA	National PKU Alliance
NSAID	nonsteroidal anti-inflammatory drug
PAH	phenylalanine hydroxylase
PAL	phenylalanine ammonia lyase
PEG	polyethylene glycol
PFS	pre-filled syringe

Abbreviation	Definition
Phe	phenylalanine
PK	pharmacokinetics
PKU	phenylketonuria
PRN	pro re nata (as needed)
rAvPAL-PEG	PEGylated recombinant Anabaena variabilis phenylalanine ammonia lyase
RCT	randomized control trial
RDI	recommended daily intake
REMS	Risk Evaluation and Mitigation Strategy
SAE	serious adverse event
SAP	Statistical Analysis Plan
sBLA	supplemental Biologics License Application
SC	Study Completion
SD	standard deviation
SoA	Schedule of Activities
SOI	Statement of Investigator
TAb	total anti-drug antibody
Tyr	tyrosine
ULN	upper limit of normal
US	United States
WOCBP	Woman of Childbearing Potential

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CLINICAL STUDY PROTOCOL

Protocol Title:	A Phase 3 Multi-Center Study to Evaluate the Safety and Efficacy of Subcutaneous Injections of Pegvaliase in Adolescent Subjects (Ages 12-17) with Phenylketonuria Featuring an Open-Label Randomized Two-Arm (Active vs Diet-Only Control) Design
Protocol Number:	165-306
Amendment Number:	Amendment 1
Compound:	pegvaliase (formerly BMN 165)
Short Title:	A Phase 3 Multi-Center Study to Evaluate the Safety and Efficacy of Subcutaneous Injections of Pegvaliase in Adolescent Subjects (Ages 12-17) with Phenylketonuria
Study Phase:	Phase 3
Sponsor Name:	BioMarin Pharmaceutical Inc.
Legal Registered Address:	105 Digital Drive Novato, CA 94949
Regulatory Agency Identifier Number(s):	IND: 076269 EudraCT: 2021-005058-27
Amendment 1 Date:	26 January 2022

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May not be divulged, published, or otherwise disclosed to others without prior written approval from BioMarin.
This study will be conducted according to the principles of Good Clinical Practice as described in the U.S. Code of Federal Regulations and the International Conference on Harmonisation Guidelines, including the archiving of essential documents.

I have read this study protocol and agree to conduct this study in compliance with the protocol, all applicable regulations, guidelines, and other information supplied to me.

Investigator Signature Date

Printed name: _____

Accepted for the Sponsor:

PI PI

PI ical Monitor Signature Date

Printed name: PI PI

Medical monitor contact information is available in Appendix 3, Section 10.3.

PROTOCOL AMENDMENT SUMMARY OF CHANGES TABLE

DOCUMENT HISTORY	
Document	**Approval date**
Amendment 1	26 January 2022
Original Protocol	22 September 2021

Amendment 1 (26 January 2022):

Overall Rationale for the Amendment

The protocol is being amended in response to feedback received from the Food and Drug Administration (FDA) in a request for advice/information letter dated 2 December 2021, and in response to feedback received from the European Medicines Agency's Paediatric Committee (PDCO) in a Paediatric Investigational Plan (PIP) modification positive opinion. Additionally, minor typographic and consistency errors are also being corrected.

Section Number and Name	Description of Change	Brief Rationale
Synopsis Figure 1.2.2 (Dosing Schematic) 4.1 (Overall Design) 5.5 (Study Stopping Criteria) 6.5 (Dose Modification) Table 6.5.1 (Study Drug Dose Titration) 7.1 (Discontinuation of Study Treatment)	Clarified that pegvaliase should be discontinued in subjects who have not achieved an adequate response (< 600 µmol/L) after 16 weeks of continuous treatment at 60 mg/day	Response to FDA's advice/information request letter dated 02 December 02 2021, per US Prescribing Information.
Synopsis 5.1 (Inclusion Criteria)	Inclusion criterion wording amended to reflect that patients should have failed both sapropterin dihydrochloride and Phe-restricted diet, not one "and/or" the other.	Response to FDA's advice/information request letter dated 02 December 02 2021
Synopsis 5.1 (Inclusion Criteria) 5.3.1 (Meals and Dietary Restrictions) 8.1.2 (Total Dietary Protein Intake, Dietary Monitoring, and Dietary Protocol Restrictions)	Added "…Inclusion criterion to include the willingness for patients to maintain stable diet under supervision of study dietician or adequately trained designee per investigator during study participation"	As per agreement with PDCO, reflected in the PIP modification approval letter (EMEA-001951-PIP01-16-M02)

Section Number and Name	Description of Change	Brief Rationale
Synopsis Table 3.1 (Objectives and Endpoints)	Additional exploratory endpoint added with regards to the comparison of subjects' optimal maintenance pegvaliase dose across different age groups within the study	As per agreement with PDCO, reflected in the PIP modification approval letter (EMEA-001951-PIP01-16-M02)
Synopsis Table 1.3.1 (SoA Part 1, Pegvaliase Arm) Table 1.3.2 (SoA Part 2, Pegvaliase Arm) Table 1.3.4 (SoA Part 2, Diet-Only Arm) 4.1 (Overall Design) 4.2 (Scientific Rationale for Study Design) 4.3 (Justification of Dose and Dose Regimen) 6.5 (Dose Modifications)	Clarified dosing administration to occur in a hospital setting for the first 8 weeks and for all subsequent dose escalations.	As per agreement with PDCO, reflected in the PIP modification approval letter (EMEA-001951-PIP01-16-M02)
Synopsis 2.2 (Background) 4.1 (Overall Design) 4.2 (Scientific Rationale for Study Design) 5.1 (Inclusion Criteria)	Added the abbreviation "US" and the eligibility age criterion (12 to 15 years (EU), inclusive) in EU	Clarify the eligibility age criterion of the patient populations for EU and US given the pegvaliase approval in US for 18 years and older and in EU for 16 years and older
5.4 (Screen Failures)	Added clarifying language around subjects who are rescreened.	Makes it clear which rescreened subjects must performed all Screening Visits procedures and assessments when re-screened.
8.4 (Pharmacokinetics) Table 1.3.1 (SoA Part 1, Pegvaliase Arm) Table 1.3.2 (SoA Part 2, Pegvaliase Arm) Table 1.3.4 (SoA Part 2, Diet-Only Arm)	Added "A pre-dose sample should be collected within 2 hours of dosing"	PK window for pre-dose is now specified to ensure consistency in the timing of the PK samples for all subjects
Synopsis 9.4.2 (Primary Endpoints)	Stratification blood Phe measurement will include 2 blood Phe measurement performed during Screening/Run-In only	The Day 1 pre-dose blood Phe sample for stratification was included in error and has been removed. The two blood Phe samples performed during Screening/Run-In are adequate for treatment stratification.

Section Number and Name	Description of Change	Brief Rationale
Table 1.3.1 (SoA Part 1, Pegvaliase Arm) Table 1.3.3 (SoA Part 1, Diet-Only Arm)	Removed "and subject appraisal of metabolic control" from footnotes.	Text had been included in error
Table 1.3.3 (SoA Part 1, Diet-Only Arm)	Tryptase measurement added at Week 73	Was omitted in error
Table 1.3.1 (SoA Part 1, Pegvaliase Arm) Table 1.3.3 (SoA Part 1, Diet-Only Arm)	Column title changed from Study Day -28/1 to Study Day -28/-1	Correcting error of missing minus (–) sign
Table 1.3.1 (SoA Part 1, Pegvaliase Arm) Table 1.3.4 (SoA Part 2, Diet-Only Arm)	Urine pregnancy test added at HRV	For consistency with Table 1.3.2
Table 1.3.1 (SoA Part 1, Pegvaliase Arm)	Complement C3/C4 measurements added at Week 13 and Week 21	For alignment with Table 1.3.4 for Week 85 and Week 96

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1 PROTOCOL SUMMARY

1.1 Synopsis

Protocol Title: A Phase 3 Multi-Center Study to Evaluate the Safety and Efficacy of Subcutaneous Injections of Pegvaliase in Adolescent Subjects (Ages 12-17) with Phenylketonuria Featuring an Open-Label Randomized Two-Arm (Active vs Diet-Only Control) Design

Short Title: A Phase 3 Multi-Center Study to Evaluate the Safety and Efficacy of Subcutaneous Injections of Pegvaliase in Adolescent Subjects (Ages 12-17) with Phenylketonuria

Study Design Rationale:

This Phase 3 multicenter study is designed to evaluate the safety and efficacy of pegvaliase administered daily to adolescent subjects (ages 12 to 17 years old (US), inclusive, and 12 to 15 years old (EU), inclusive) with phenylketonuria (PKU). Subjects will be randomized in a 2:1 ratio to the active (pegvaliase) and control (diet-only) treatment arms, respectively, with 36 subjects receiving pegvaliase and 18 subjects managing their PKU with diet alone. The primary efficacy endpoint is the change from baseline in blood phenylalanine (Phe) following 72 weeks on study (the end of Part 1). Beginning Week 73, subjects in the active (pegvaliase) treatment arm will continue to receive pegvaliase in the Extension Phase (Part 2) for up to an additional 80 weeks (through Week 153). Subjects in the control (diet only) treatment arm will initiate pegvaliase treatment beginning Week 73 and, from Weeks 73 through 145, follow the same dosing and assessment schedule that the active subjects followed from Weeks 1 through 73, after which they will receive pegvaliase for up to 8 additional weeks (through Week 153).

A randomized control trial (RCT) design, with diet-only management of PKU as the control, will allow for robust data collection and minimize the risk of confounding factors influencing the results. An open-label diet-only control arm is the best comparator for this age group as daily placebo injections would place undue burden on adolescent PKU patients.

In Part 1 (through the Week 73 visit), randomized subjects in both the control (diet-only) and active (pegvaliase) treatment arms will be required to maintain the same level of protein intake they established during Screening/Run-in. In Part 2, subjects in the control (diet-only) arm must continue to maintain this prescribed level of protein intake, whereas subjects randomized to the active (pegvaliase) arm whose blood Phe concentration is ≤ 360 µmol/L for a minimum of 4 weeks may adjust dietary protein intake based on individual subject

response to pegvaliase and guidance from the investigator. Subjects are required to maintain a consistent level of protein intake during the study to ensure that the safety and efficacy endpoints are attributable to pegvaliase rather than to changes in dietary protein intake.

Pegvaliase will be subcutaneously injected in a hospital setting for the first 8 weeks and thereafter at the clinic or at home by a trained subject, caregiver, adult observer, or visiting healthcare provider. Pegvaliase will also be injected in a hospital setting for any subsequent dose escalations. Administration at home makes daily treatment less burdensome for subjects, thereby improving treatment compliance.

An adult observer is required for a minimum of 1 hour post injection throughout the study. An adult observer is defined as someone who will be present with the subject during and after pegvaliase administration, is able to recognize the signs and symptoms of an anaphylaxis episode, and is able to call for emergency medical support and administer epinephrine, if warranted. The requirement for an adult observer is included to ensure that subjects self-administering pegvaliase would have support in recognizing and quickly responding to signs and symptoms of anaphylaxis.

The primary efficacy endpoint is the change from treatment naïve baseline in blood Phe following 72 weeks on study (the end of Part 1). Blood Phe level is the established clinical marker used to diagnose PKU and assess disease control, and reduction of blood Phe concentration to target levels is the universally acknowledged treatment goal for PKU patients.

Objectives and Endpoints:

The primary, secondary, and exploratory objectives and associated endpoints are shown below. Refer to the Schedule of Activities (SoA) for the complete listing of assessment timepoints.

Objectives	Endpoints
Primary	
To evaluate the efficacy of pegvaliase in adolescent subjects with PKU	The primary efficacy endpoint is change in blood Phe concentration from treatment naïve baseline following 72 weeks on study. Change in blood Phe from baseline is compared between subjects in the active (pegvaliase) and the control (diet-only) treatment arms.
To evaluate the safety of pegvaliase in adolescent subjects with PKU	• Adverse events (AEs), including serious AEs (SAEs) • Clinical laboratory tests (chemistry, hematology, and urinalysis) results • Vital signs • Physical examination (including height, weight) • Electrocardiogram (ECG) test results • Immunogenicity test results (anti-PEG IgG, anti-PEG IgM, anti-PAL IgG, anti-PAL IgM, Tab, Nab, and anti-pegvaliase IgE [hypersensitivity reaction visits only]).
Secondary	
To characterize total dietary protein intake in adolescent subjects with PKU after pegvaliase treatment	Total dietary protein intake must remain stable for change in blood Phe, the primary endpoint, to be accurately assessed. To gauge the stability of total protein intake, the change from baseline at Week 73 in total dietary protein intake (defined as the sum of the protein intake from medical food and intact food) will be measured.
To characterize the pharmacokinetics (PK) of pegvaliase in adolescent subjects with PKU	Trough plasma PK samples will be collected and analyzed as specified in the SoA. PK parameters assessed with intensive PK sampling will include $AUC_{0\text{-}24hr,ss}$, $C_{max,ss}$, $T_{max,ss}$, $t_{1/2}$, $CL_{ss/F}$, and $V_{dz/F}$. For subjects randomized to the active (pegvaliase) treatment arm, intensive PK sampling will be performed at Week 73 and for dose escalations to 20, 40, and 60 mg/day. For subjects randomized to the control (diet-only) treatment arm, there are no PK assessments in Part 1. In Part 2, intensive PK sampling will be performed at Week 145 and for dose escalations to 20, 40, and 60 mg/day.

Objectives	Endpoints
Exploratory Objectives	
To evaluate the effect of pegvaliase treatment on neurocognitive outcomes in adolescent subjects with PKU.	• The ADHD Rating Scale IV inattention subscale (ADHD-RS IV) rated by the parent/guardian and performed at Baseline (Day 1, pre-dose) and every 12 weeks throughout the study. • The Behavior Rating Inventory of Executive Function (BRIEF) rated by the parent/guardian and performed at Baseline (Day 1, pre-dose) and every 12 weeks throughout the study.
To compare subjects' optimal maintenance pegvaliase dose across different age groups within the study	• Time to subjects' optimal maintenance dose compared to older age subsets • Number of subjects who require the highest (60 mg/day) maintenance dose • Percentage/number of subjects in the blood Phe target ranges of < 600 µmol/L and < 360 µmol/L.
Explore the biochemical, molecular, and cellular aspects of PKU.	Blood will be collected to evaluate biochemical, molecular, and cellular aspects of PKU and to develop the assays used for these evaluations.
PAH genotyping	Testing will be performed on samples collected Day 1 pre-dose where allowed by local health authorities and the EC/IRB.

Overall Design:

Study 165-306 is an open-label, two-arm, randomized control evaluation of pegvaliase in approximately 54 subjects (ages 12 to 17 years old (US), inclusive, and 12 to 15 years old (EU), inclusive) with diet only as the control arm. To be eligible for the study, potential subjects must meet all eligibility requirements described in the protocol, including the following selected key inclusion criteria:

- Is 12 to 17 years old (US), inclusive, or 12 to 15 years old (EU), inclusive, at the start of the Screening/Run-in Period (Day -28).
- Diagnosis of PKU and failure to maintain recommended blood Phe levels on existing management (sapropterin dihydrochloride and Phe-restricted diet) demonstrated by 2 blood Phe concentration measurements > 600 µmol/L during the Screening/Run-in Period (7 to 10 days between blood Phe assessments) and average blood Phe concentration > 600 µmol/L over the past 12 months (per available data).
- Willing and able to maintain and adjust dietary and medical protein food intake according to the study protocol and under the supervision of study dietician or adequately trained designee per investigator during study participation.

- An adult (≥ 18 years of age) has been identified who is willing and competent to observe the subject during study drug administration and for a minimum of 1 hour following administration. A home healthcare nurse may perform the study drug observations consistent with local regulatory and site requirements.

Selected key exclusion criteria include the following:

- Potential subjects are not eligible if they are unable to identify and/or communicate to others that they are experiencing symptoms of potential anaphylaxis due to cognitive impairment or other reasons.

Following enrollment, subjects will be randomized 2:1 to the active (pegvaliase) and control (diet-only) treatment arms, with 36 subjects receiving daily pegvaliase and 18 subjects managing PKU by diet alone. Treatment assignment will be stratified by Screening/Run-In blood Phe (mean of the 2 pre-treatment blood Phe assessments performed during the Screening/Run-in period) of ≤ 1000 µmol/L or > 1000 µmol/L. The primary efficacy endpoint is the change from baseline blood Phe following 72 weeks on study (the end of Part 1). Subjects in the active and control arms will follow the same visit schedules and perform the same assessments throughout the 72-week Primary Treatment Phase (Part 1) except that the control subjects will not be receiving pegvaliase so will not have PK draws or immunogenicity assessments.

After providing informed consent, subjects will enter the Screening/Run-in period, which will last 4 weeks and include 2 assessments of blood Phe levels, 7 to 10 days apart, to determine eligibility. Following confirmation that the 2 blood Phe results are > 600 µmol/L and all other eligibility criteria have been met, subjects may be enrolled in the study on Day 1. During the Screening/Run-in and the Primary Treatment Phase (Part 1), subjects will be required to maintain stable protein intake from both medical food and/or intact food.

The duration of Part 1 is 72 weeks, during which pegvaliase treatment will be initiated for subjects in the active arm using an Induction/Titration/Maintenance (I/T/M) dosing regimen. The I/T/M dosing regimen consists of Induction/Titration Dosing (9 weeks) and Maintenance Dosing. Required hospital setting visits during Part 1 will occur for all dosing visits for the first 8 weeks, every 4 weeks thereafter, and when the pegvaliase dose is escalated to 20, 40, or 60 mg/day. The assessments performed at the clinic visits include measurement of blood Phe, neurocognitive assessments, PK blood draws (active subjects only), and safety assessments.

Beginning Week 73, subjects in the active treatment arm will continue to receive pegvaliase in the Extension Phase (Part 2) for up to 80 additional weeks (through Week 153). Beginning Week 73, subjects in the control treatment arm will initiate pegvaliase treatment and follow a

parallel schedule for dosing, study visits, and assessments in Weeks 73 through 145 to that followed by the active arm subjects during the Primary Treatment Phase (Part 1) from Weeks 1 through 73, after which they will receive pegvaliase for up to 8 additional weeks (through Week 153).

Brief Summary:

The purpose of Study 165-306 is to evaluate the safety and efficacy of pegvaliase and characterize the PK of pegvaliase in adolescent subjects with PKU. Study details include:

Condition/Disease: PKU is a rare autosomal recessive genetic disorder caused by mutations in the phenylalanine hydroxylase (PAH) gene, leading to an absence or deficiency in PAH enzyme activity and subsequent elevation of the amino acid phenylalanine (Phe) in the blood, known as hyperphenylalaninemia (HPA) (Vockley 2014; Mitchell 2011). Deficiency of PAH results in an abnormally elevated concentration of Phe, which is toxic to the brain. High Phe levels during infancy and early childhood cause profound neurocognitive and developmental defects, and poorly controlled blood Phe levels in older children and adults are associated with learning disabilities, attention deficit hyperactivity disorder, behavioral problems, and psychiatric symptoms.

Study Hypothesis: The study hypothesis is that subjects receiving pegvaliase will show a greater reduction in blood Phe following 72 weeks on study compared to subjects in the diet-only control group.

Study Duration: The study duration is up to 160 weeks for subjects randomized to active (pegvaliase) treatment. This includes a 4-week Screening/Run-in period, up to 152 weeks of pegvaliase treatment in Parts 1 and 2, and the Study Completion/Early Termination (SC/ET) visit 4 weeks after the final dose of pegvaliase. The study duration is also 160 weeks for subjects randomized to control (diet-only) treatment. This includes a 4-week Screening/Run-in period, 72 weeks managing PKU with diet alone while following the Part 1 assessment schedule, 72 weeks of pegvaliase treatment following the same Part 1 assessment schedule, 8 additional weeks of pegvaliase treatment, and the SC/ET visit 4 weeks after the final dose of pegvaliase.

Treatment Duration: The pegvaliase treatment duration will be 152 weeks for subjects randomized to the active (pegvaliase) treatment arm (n=36). The pegvaliase treatment duration will be 80 weeks for subjects randomized to the control (diet-only) treatment arm (n=18).

Health Measurement/Observation: Reduction in blood Phe.

Visit Frequency: For both active and control subjects, hospital setting visits in Part 1 will occur for all dosing visits for the first 8 weeks, then every 4 weeks through Week 73 and for all dose escalations. For subjects randomized to active (pegvaliase) treatment, clinic visits in Part 2 will occur every 4 weeks through Week 153 and for all dose escalations. For subjects randomized to control (diet-only) treatment, hospital setting visits in Part 2 will occur for all dosing visits for the first 8 weeks, then every 4 weeks through Week 153. For subjects randomized to active (pegvaliase) treatment in Part 1 and subjects randomized to control (diet-only) treatment in Part 2, hospital setting visits will also occur when their dose level is escalated to 20, 40, and 60 mg/day.

Number of Subjects:

Approximately 54 subjects will be enrolled and randomized to the pegvaliase or diet-only groups in a 2:1 ratio. Assuming the mean (SD) reduction in Phe following 72 weeks on study is 640 (570) µmol/L in the pegvaliase group and 100 (250) µmol/L in the diet-only group, a total sample size of 54 subjects will provide more than 90% power to detect a treatment difference, based on the two-sample T-test with unequal variance and a significance level of 0.05 (two sided). The assumption of treatment effect is based on clinical data from previous pegvaliase studies and a PKU registry study. See the Statistical Analysis Plan (SAP) for additional details on the sample size calculation.

Approximately 72 subjects will be screened for study participation such that approximately 54 subjects will be enrolled in the study.

Note: Potential subjects who are screened for the purpose of determining eligibility for the study, but do not participate in the study, are not considered enrolled.

Treatment Groups and Duration:

The duration of the Primary Treatment Phase (Part 1) is 72 weeks, during which pegvaliase treatment will be initiated for subjects randomized to the active (pegvaliase) treatment arm using an I/T/M dosing regimen. Induction will consist of a 4-week period when subjects randomized to active drug will receive pegvaliase subcutaneously with a pre-filled syringe (PFS) at a fixed dose of 2.5 mg/week and during which the dosing regimen will not be modified in response to blood Phe concentration. Once subjects complete induction they will titrate up to a dose of 10 mg/day. The dosing frequency will be increased gradually during titration dosing to daily (7 days/week). During Maintenance Dosing the dose will be increased to 20 mg/day, 40 mg/day, and 60 mg/day depending upon individual blood Phe lowering efficacy (per the investigator's discretion). The dose should be increased to 40 mg/day if blood Phe is > 360 µmol/L after 24 weeks dosing at 20 mg/day and increased to

60 mg/day if blood Phe is > 360 µmol/L after 16 weeks dosing at 40 mg/day. If a subject's blood Phe is > 600 µmol/L after 16 weeks of continuous treatment with pegvaliase at 60 mg/day, pegvaliase should be permanently discontinued. BioMarin recommends that the target blood Phe levels should be based on individual needs, with a target blood Phe ≤ 600 µmol/L or lower. During the Primary Treatment Phase (Part 1), subjects randomized to the control (diet-only) treatment arm will have their PKU managed by diet alone.

Following completion of Part 1, subjects randomized to active (pegvaliase) treatment will enter the Extension Phase (Part 2), during which subjects will continue dosing with pegvaliase at their optimal maintenance dose level for up to an additional 80 weeks (through Week 153) in order to evaluate the long-term safety and efficacy of pegvaliase in adolescent subjects with PKU. Following completion of Part 1, subjects randomized to control (diet-only) treatment will repeat the Part 1 assessment schedule while initiating pegvaliase dosing from Weeks 73 through 145, after which they will receive pegvaliase for up to 8 additional weeks (through Week 153).

Data Monitoring Committee:

An independent Data Monitoring Committee (DMC) will monitor the safety of study subjects. The DMC is an independent committee that acts in an advisory capacity to BioMarin.

1.2 Study Schema

Figure 1.2.1: Active (Pegvaliase) vs Control (Diet-Only) Treatment Arms (Randomized Treatment Assignment)

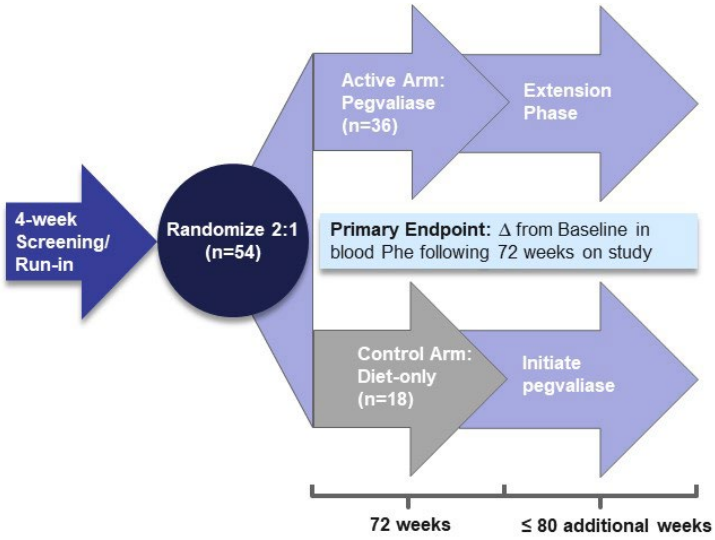
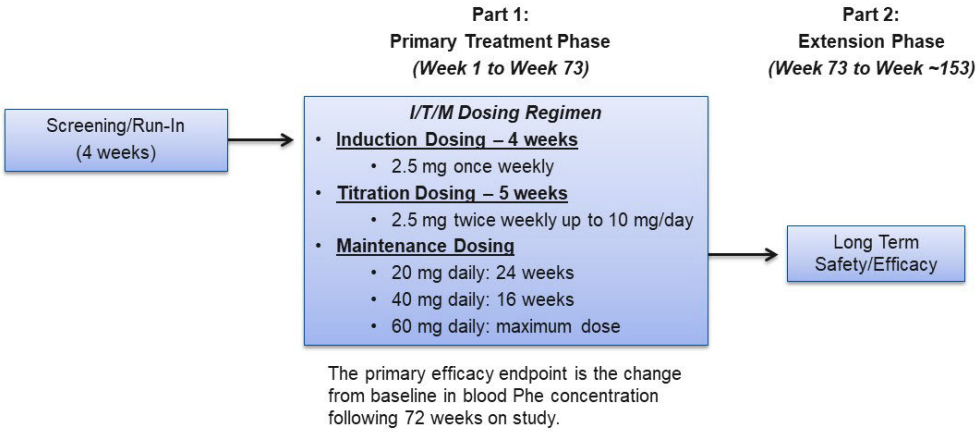


Figure 1.2.2: Dosing Schematic for Active (Pegvaliase) Treatment Arm



Note: The 18 control (diet-only) subjects will follow the Part 1 assessment schedule (except for pegvaliase dosing and PK/immunogenicity assessments) from Weeks 1 through Week 73 and then repeat the Part 1 assessment schedule including pegvaliase dosing and PK/immunogenicity assessments from Week 73 through 145, after which they will receive pegvaliase for 8 additional weeks (through Week 153).

Prior to dose escalation, the minimum amount of time pegvaliase can be dosed at 20 mg/day before escalating is 24 weeks and the minimum amount of time at 40 mg/day before escalating is 16 weeks. The dose should be increased to 40 mg/day if blood Phe is > 360 µmol/L after 24 weeks dosing at 20 mg/day, and increased to 60 mg/day if blood Phe is > 360 µmol/L after 16 weeks dosing at 40 mg/day. If a subject's blood Phe is > 600 µmol/L after 16 weeks of continuous treatment with pegvaliase at 60 mg/day, pegvaliase should be permanently discontinued.

1.3 Schedule of Activities (SoA)

Table 1.3.1: Schedule of Activities for Part 1 (Subjects Randomized to Pegvaliase Treatment Arm)

Assessment [a]	Study Week	Screen[b]	1	2	3	4	5	6	7	8	9	13	17	21	25	29	33	37	41	45	49	53	57	61	65	69	73	HRV[c]	SC/ET[d]
	Study Day	-28/-1	1	8	15	22	29	36	43	50	57	85	113	141	169	197	225	253	281	309	337	365	393	421	449	477	505		
	Window (Days)			±3	±3	±3	±3	±3	±3	±3	±3	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7		
Informed consent		X																											
Drug administration training [e]			X																										
Study drug observer training [f]		X																											
Demographics		X																											
Medical history [g]		X																											
HIV, hepatitis B and C screens [h]		X																											
PAH genotyping [i]			X																										
12 lead ECG		X	X																										X
Physical examination [j]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Weight		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Height		X	X				X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
Vital signs		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Clinical safety laboratory tests [k]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
C-reactive protein		X	X								X		X		X		X		X		X		X		X		X	X	X
Erythrocyte sedimentation rate [l]		X	X																										X
Serum cortisol [m]			X												X						X						X		X
Complements C3 and C4			X				X				X	X	X	X	X		X		X		X		X		X		X	X	X
Tryptase			X																									X	
Urine albumin/creatinine ratio [n]			X								X		X		X		X		X		X		X		X		X	X	X
Urine pregnancy test [o]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
3-day diet diary [p]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
PK (plasma pegvaliase) [q]			X				X				X	X	X	X	X		X		X		X		X		X		X		X

Assessment [a]	Study Week	Screen[b]	1	2	3	4	5	6	7	8	9	13	17	21	25	29	33	37	41	45	49	53	57	61	65	69	73	HRV[c]	SC/ET[d]
	Study Day	-28/-1	1	8	15	22	29	36	43	50	57	85	113	141	169	197	225	253	281	309	337	365	393	421	449	477	505		
	Window (Days)			±3	±3	±3	±3	±3	±3	±3	±3	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7		
Blood for exploratory analyses [i]			X				X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
Blood Phe, tyrosine [r]		X [b]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
Immunogenicity assessments [s]			X	X	X	X	X	X	X	X	X	X	X	X	X		X		X		X		X		X		X	X	X
ADHD-RS IV [t]			X									X			X			X			X			X			X		X
BRIEF [u]			X									X			X			X			X			X			X		X
Weekly call (non-visit weeks) [v]																													
Adverse events [w]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant medication [w]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Administer study drug [x]			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		

ACTH, adrenocorticotropic hormone; ADHD-RS IV, attention deficit hyperactivity disorder rating scale IV; BRIEF, Behavior Rating Inventory of Executive Function; EC/IRB, Ethics Committee/Institutional Review Board; ECG, electrocardiogram; eCRF, electronic Case Report Form; HRV, hypersensitivity reaction visit; NSAID, non-steroidal anti-inflammatory drug; PAH, phenylalanine hydroxylase; PE, physical examination; PK, pharmacokinetics; PKU, phenylketonuria; SC/ET, Study Completion/Early Termination visit.

[a] Scheduled visits take place at the hospital setting (for all dosing visits for the first 8 weeks, and for all dose escalation visits), clinic, or, with the approval of the investigator or designee, virtually. For virtual visits the investigator/designee performs assessments while online with the subject, apart from specimen collections and vital signs, which are performed by home healthcare personnel. Non-clinic visits are via telephone. Assessments are performed pre-dose unless otherwise specified.

[b] After written informed consent, screening assessments must be performed within 28 days prior to Part 1, Day 1. Subjects will be assessed for blood Phe concentration during Screening/Run-in with 2 measurements 7 to 10 days apart. These 2 blood Phe measurements should be scheduled to occur: (1) in the Day -25 to Day -23 window; and (2) 7 to 10 days later (ie, in the Day -18 to Day -13 window).

[c] If the investigator determines that the NCI-CTCAE Grade ≥ 3 hypersensitivity reaction is related to administration with study drug, the subject will be asked to return to the clinic within 24 hours of event onset for a hypersensitivity reaction visit (HRV). Sampling for anti-pegvaliase IgE should be performed 8 to 24 hours after event onset and before the next dose of study drug.

[d] Subjects should perform the Study Completion (SC) Visit 4 weeks after the last dose of study drug or after completion of study participation, whichever occurs last. For subjects who terminate from the study early, the Early Termination (ET) Visit occurs within 4 weeks of the last study drug dose or after withdrawal from study participation, whichever occurs last. Subjects who discontinue from study drug early will be asked to continue to complete the remaining study assessments until study completion as long as such continued participation will not detrimentally affect the health, safety, and welfare of the subject per investigator determination.

[e] Once subject/caregiver competence is documented, the subject/caregiver administers study drug daily.

[f] Must be completed prior to administration of the first dose of study drug. Additional observers identified after screening must be trained.

[g] PKU history includes highest blood Phe (and age).

[h] Subjects with documented negative results within the last 4 weeks do not need to be retested.

[i] Sample collection should be performed where allowed by country-specific regulations and EC/IRB.

[j] A full physical examination (PE) will be performed at Screening, Baseline, Week 73, and the SC/ET visit. A brief PE will be performed at other visits. A full PE will include, at a minimum, general appearance (head, eyes, ears, nose, and throat) and an assessment of cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, musculoskeletal, and neurologic systems. A brief PE may be performed at the discretion of the investigator based on the subject's clinical condition and may include, but is not limited to, general appearance and an assessment of cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, musculoskeletal, and neurologic systems.

[k] It is recommended that urine samples are obtained as a first or second morning void. In the event of elevated urinary protein on a test result, a repeat urinalysis should be performed. This repeat urine sample must be performed in the morning at the first or second morning void to allow for accurate test results and may be performed by a home healthcare nurse.

[l] To be done by a local laboratory.

[m] Serum cortisol samples should be taken before study drug administration in the morning before 10:00 am. A home healthcare nurse may collect samples for subject convenience. If 2 results are low and abnormal, the subject will be asked to perform additional sampling for plasma ACTH and a low-dose conventional ACTH stimulation test or the ACTH test method based on the clinical practice at the site.

[n] It is recommended that urine samples be obtained as a first or second morning void. Subjects with a confirmed urine/albumin creatinine ratio of ≥ 100 mg/g should be referred to a nephrologist for consultation if results were within normal range at Baseline. Subjects who had elevated results at Baseline followed by a confirmed subsequent increase of 100-200 mg/g from Baseline should also be referred to a nephrologist for consultation.

[o] To be done locally (if applicable). If urine pregnancy test is positive or equivocal, serum pregnancy test (central laboratory) must be done.

[p] Subjects should record at home all food, beverages, special low-protein foods, and medical foods consumed during the 3 consecutive days preceding a study visit. No change in protein (dietary and medical food) intake is allowed in Part 1. During Screening/Run-in, 3-day diet diary assessments are performed 3 times, each time covering the 3-day periods prior to blood Phe assessments (and collected on the day of those assessments). The 3 Screening/Run-in blood Phe assessments are scheduled to occur (1) at Day -25 to Day -23; (2) 7 to 10 days later (ie, in the Day -18 to Day -13 window); and (3) at Day 1 pre-dose. Thus, the 3 day diet assessments will assess diet for (1) the 3 days within the Day -28 to Day -23 window immediately prior to the first blood Phe assessment; (2) the 3 days within the Day -21 to Day -15 window immediately prior to the second blood Phe assessment; and (3) for Day -3 to Day 1.

[q] Blood is collected before study drug administration (pre-dose) within 2 hours of dosing. Intensive PK sampling will be performed at Week 73 and at dose escalation visits in all subjects. Samples will be taken at pre-dose (within 2 hours of dosing), 2, 4, 8, 12, and 24 hours post dose. The 24-hour sample will be taken prior to the next daily dose.

[r] Blood is collected for blood Phe analysis after fasting a minimum of 2.5 hours. In addition to the blood draws for blood Phe analysis collected in the clinic and shown in the SoA, there are 5 required blood collections that may be drawn either at the clinic or elsewhere (eg, at home). These are to be collected at Weeks 11, 15, and 19, and 2 weeks prior to the planned escalations to 40 and 60 mg/day.

[s] Immunogenicity assays include total anti-pegvaliase antibodies (TAb), anti-PAL IgG, anti-PAL IgM, anti-PEG IgM, anti-PEG IgG, and neutralizing antibodies (NAb). At the HRV, only anti-pegvaliase IgE will be assessed.

[t] Completed by parent/guardian.

[u] Completed by parent/guardian.

[v] Between scheduled clinic visits, the clinic staff will contact the subject weekly to monitor if the subject is experiencing problems with drug administration, to ask about any AEs or concomitant medications, and to answer questions.

[w] AEs and concomitant medications should be noted whenever a subject is assessed by study personnel. Following signed informed consent and prior to the first dose of pegvaliase, only SAEs associated with study procedures are collected. After the first dose all AEs and SAEs are collected until 4 weeks after the last study drug dose or the Early Termination Visit, whichever occurs last. If there is a severe (Grade 3) injection site reaction, the subject may be referred to a dermatologist for consultation and a skin biopsy (optional; if clinically indicated). If photographs of the severe injection site reaction are taken, they may be collected by the sponsor.

[x] Subjects must be premedicated with an H1 antagonist, and an H2 antagonist, and an antipyretic approximately 2 to 3 hours prior to each dose of study drug until they reach the Maintenance Phase, defined as when subjects achieve blood Phe ≤ 600 µmol/L while they are on a stable dose of pegvaliase. Premedication may be considered during the Maintenance Phase at the investigator's discretion, eg, approximately 2 to 3 hours prior to study drug for 1 week upon reintroduction of study drug, upon resolution of an AE, following any dose interruption of ≥ 4 days, and for any dose increases in the Extension Phase. Subjects may also be premedicated at any other time in the study per investigator discretion. When an NSAID is administered as a premedication, it should be given with food. For non-clinic visits, subjects are asked about study drug administration.

Table 1.3.2: Schedule of Activities for Part 2 (Subjects Randomized to Pegvaliase Treatment Arm)

Assessment [a]	Study Week	73[b]	77	81	85	89	93	97	101	105	109	113	117	121	125	129	133	137	141	145	149	153	HRV[c]	SC/ET[d]
	Study Day	505	533	561	589	617	645	673	701	729	757	785	813	841	869	897	925	953	981	1009	1037	1065		
	Window (Days)	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7		
Study drug observer training [e]																								
12 lead ECG																								X
Physical examination [f]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Weight	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Height	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
Vital signs	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Clinical safety laboratory tests [g]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
C-reactive protein	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Erythrocyte sedimentation rate [h]																								X
Serum cortisol [i]	X						X						X								X			X
Complements C3 and C4	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Tryptase																							X	
Urine albumin/creatinine ratio [j]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Urine pregnancy test [k]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
3-day diet diary [l]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
PK (plasma pegvaliase) [m]	X		X		X		X		X		X		X		X		X		X		X			X
Blood for exploratory analyses [n]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
Blood Phe, tyrosine [o]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Immunogenicity assessments [p]	X		X		X		X		X		X		X		X		X		X		X	X	X	X
ADHD-RS IV [q]	X			X			X			X			X			X			X					X
BRIEF [r]	X			X			X			X			X			X			X					X
Weekly call (non-visit weeks) [s]																								
Adverse events [t]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant medication [t]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

Assessment [a]	Study Week	73[b]	77	81	85	89	93	97	101	105	109	113	117	121	125	129	133	137	141	145	149	153	HRV[c]	SC/ET[d]
	Study Day	505	533	561	589	617	645	673	701	729	757	785	813	841	869	897	925	953	981	1009	1037	1065		
	Window (Days)	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7		
Administer study drug [u]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		

ACTH, adrenocorticotropic hormone; ADHD-RS IV, attention deficit hyperactivity disorder rating scale IV; BRIEF, Behavior Rating Inventory of Executive Function; ECG, electrocardiogram; eCRF, electronic Case Report Form; HRV, hypersensitivity reaction visit; PE, physical examination; PK, pharmacokinetics; PKU, phenylketonuria; NSAID, non-steroidal anti-inflammatory drug; SC/ET, Study Completion/Early Termination visit.

[a] Scheduled visits take place at the hospital setting (for all dose escalation visits), clinic, or, with the approval of the investigator or designee, virtually. For virtual visits the investigator/designee performs assessments while online with the subject, apart from specimen collections and vital signs, which are performed by home healthcare personnel. Non-clinic visits are via telephone. Assessments are performed pre-dose unless otherwise specified.

[b] The Week 73 visit marks both the end of the Primary Treatment Phase (Part 1) and the beginning of the Extension Phase (Part 2). The pre-dose assessments constituting the end of the Primary Treatment Phase and the administration of study drug constituting the beginning of the Extension Phase are shown in the Week 73 columns of both Table 1.3.1 and Table 1.3.2.

[c] If the investigator determines that the NCI-CTCAE Grade ≥ 3 hypersensitivity reaction is related to administration with study drug, the subject will be asked to return to the clinic within 24 hours of event onset for a hypersensitivity reaction visit (HRV). Sampling for anti-pegvaliase IgE should be performed 8 to 24 hours after event onset and before the next dose of study drug.

[d] Subjects should perform the Study Completion (SC) Visit 4 weeks after the last dose of study drug or after completion of study participation, whichever occurs last. For subjects who terminate from the study early, the Early Termination (ET) Visit occurs within 4 weeks of the last study drug dose or after withdrawal from study participation, whichever occurs last. Subjects who discontinue from study drug early will be asked to continue to complete the remaining study assessments until study completion as long as such continued participation will not detrimentally affect the health, safety, and welfare of the subject per investigator determination.

[e] Additional observers identified after screening must be trained.

[f] A full PE will be performed at Week 73 and the SC/ET visit. A brief PE will be performed at other visits. A full PE will include, at a minimum, general appearance (head, eyes, ears, nose, and throat) and an assessment of cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, musculoskeletal, and neurologic systems. A brief PE may be performed at the discretion of the investigator based on the subject's clinical condition and may include, but is not limited to, general appearance and an assessment of cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, musculoskeletal, and neurologic systems.

[g] It is recommended that urine samples are obtained as a first or second morning void. In the event of elevated urinary protein on a test result, a repeat urinalysis should be performed. This repeat urine sample must be performed in the morning at the first or second morning void to allow for accurate test results and may be performed by a home healthcare nurse.

[h] To be done by a local laboratory.

[i] Serum cortisol samples should be taken before study drug administration in the morning before 10:00 am. A home healthcare nurse may collect samples for subject convenience. If 2 results are low and abnormal, the subject will be asked to perform additional sampling for plasma ACTH and a low-dose conventional ACTH stimulation test or the ACTH test method based on the clinical practice at the site.

[j] It is recommended that urine samples be obtained as a first or second morning void. Subjects with a confirmed urine/albumin creatinine ratio of ≥ 100 mg/g should be referred to a nephrologist for consultation if results were within normal range at Baseline. Subjects who had elevated results at Baseline followed by a confirmed subsequent increase of 100-200 mg/g from Baseline should also be referred to a nephrologist for consultation.

[k] To be done locally (if applicable). If urine pregnancy test is positive or equivocal, serum pregnancy test (central laboratory) must be done.

[l] Subjects should record at home all food, beverages, special low-protein foods, and medical foods consumed during the 3 consecutive days preceding a study visit. No change in protein (dietary and medical food) intake is allowed in Part 1.

[m] Blood is collected before study drug administration (pre-dose) within 2 hours of dosing. Intensive PK sampling will be performed at Week 73 and at dose escalation visits in all subjects. Samples will be taken at pre-dose (within 2 hours of dosing), 2, 4, 8, 12, and 24 hours post dose. The 24-hour sample will be taken prior to the next daily dose.

[n] Sample collection should be performed where allowed by country-specific regulations and EC/IRB.

[o] Blood is collected for blood Phe analysis after fasting a minimum of 2.5 hours.

[p] Immunogenicity assays include total anti-pegvaliase antibodies (TAb), anti-PAL IgG, anti-PAL IgM, anti-PEG IgM, anti-PEG IgG, and neutralizing antibodies (NAb). At the HRV, only anti-pegvaliase IgE will be assessed.

[q] Completed by parent/guardian.

[r] Completed by parent/guardian.

[s] Between scheduled clinic visits, the clinic staff will contact the subject weekly to monitor if the subject is experiencing problems with drug administration, to ask about any AEs or concomitant medications, and to answer questions.

[t] AEs and concomitant medications should be noted whenever a subject is assessed by study personnel. All AEs and SAEs are collected until 4 weeks after the last study drug dose or the Study Completion Visit/Early Termination visit, whichever occurs last. If there is a severe (Grade 3) injection site reaction, the subject may be referred to a dermatologist for consultation and a skin biopsy (optional; if clinically indicated). If photographs of the severe injection site reaction are taken, they may be collected by the sponsor.

[u] Subjects must be premedicated with an H1 antagonist, and an H2 antagonist, and an antipyretic approximately 2 to 3 hours prior to each dose of study drug until they reach the Maintenance Phase, defined as when subjects achieve blood Phe ≤ 600 µmol/L while they are on a stable dose of pegvaliase. Premedication may be considered during the Maintenance Phase at the investigator's discretion, eg, approximately 2 to 3 hours prior to study drug for 1 week upon reintroduction of study drug, upon resolution of an AE, following any dose interruption of ≥ 4 days, and for any dose increases in the Extension Phase. Subjects may also be premedicated at any other time in the study per investigator discretion. When an NSAID is administered as a premedication, it should be given with food. For non-clinic visits, subjects are asked about study drug administration.

Table 1.3.3: Schedule of Activities for Part 1 (Subjects Randomized to Diet-Only Treatment Arm)

Assessment [a]	Study Week	Screen[b]	1	2	3	4	5	6	7	8	9	13	17	21	25	29	33	37	41	45	49	53	57	61	65	69	73	
	Study Day	-28/-1	1	8	15	22	29	36	43	50	57	85	113	141	169	197	225	253	281	309	337	365	393	421	449	477	505	SC/ET[c]
	Window (Days)			±3	±3	±3	±3	±3	±3	±3	±3	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	
Informed consent		X																										
Drug administration training [d]																											X	
Study drug observer training [e]																											X	
Demographics		X																										
Medical history (allergy, PKU) [f]		X																										
HIV, hepatitis B and C screens [g]		X																										
PAH genotyping [h]			X																									
12 lead ECG		X	X																								X	X
Physical examination [i]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Weight		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Height		X	X				X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital signs		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Clinical safety laboratory tests [j]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
C-reactive protein		X	X								X		X		X		X		X		X		X		X		X	X
Erythrocyte sedimentation rate [k]		X	X																								X	X
Serum cortisol [l]			X												X						X						X	X
Complements C3 and C4			X				X				X		X		X		X		X		X		X		X		X	X
Tryptase			X																								X	
Urine albumin/creatinine ratio [m]			X								X		X		X		X		X		X		X		X		X	X
Urine pregnancy test [n]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
3-day diet diary [o]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Blood for exploratory analyses [h]			X				X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Blood Phe, tyrosine [p]		X [b]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
ADHD-RS IV [q]			X									X			X			X			X			X			X	X

	Study Week	Screen[b]	1	2	3	4	5	6	7	8	9	13	17	21	25	29	33	37	41	45	49	53	57	61	65	69	73	
	Study Day	-28/-1	1	8	15	22	29	36	43	50	57	85	113	141	169	197	225	253	281	309	337	365	393	421	449	477	505	SC/ET[c]
Assessment [a]	Window (Days)			±3	±3	±3	±3	±3	±3	±3	±3	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	
BRIEF [r]			X									X			X			X			X			X			X	X
Weekly call (non-visit weeks) [s]																												
Adverse events [t]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant medication [t]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

ACTH, adrenocorticotropic hormone; ADHD-RS IV, attention deficit hyperactivity disorder rating scale IV; BRIEF, Behavior Rating Inventory of Executive Function; EC/IRB, Ethics Committee/Institutional Review Board; ECG, electrocardiogram; eCRF, electronic Case Report Form; HRV, hypersensitivity reaction visit; PAH, phenylalanine hydroxylase; PE, physical examination; PK, pharmacokinetics; PKU, phenylketonuria; SC/ET, Study Completion/Early Termination visit.

[a] Scheduled visits take place at the clinic or, with the approval of the investigator or designee, virtually. For virtual visits the investigator/designee performs assessments while online with the subject, apart from specimen collections and vital signs, which are performed by home healthcare personnel. Non-clinic visits are via telephone.

[b] After written informed consent, screening assessments must be performed within 28 days prior to Part 1, Day 1. Subjects will be assessed for blood Phe concentration during Screening/Run-in with 2 measurements 7 to 10 days apart. These 2 blood Phe measurements should be scheduled to occur: (1) in the Day -25 to Day -23 window; and (2) 7 to 10 days later (ie, in the Day -18 to Day -13 window).

[c] Subjects should perform the Study Completion (SC) Visit 4 weeks after the last dose of study drug or after completion of study participation, whichever occurs last. For subjects who terminate from the study early, the Early Termination (ET) Visit occurs within 4 weeks of the last study drug dose or after withdrawal from study participation, whichever occurs last. Subjects who discontinue from study drug early will be asked to continue to complete the remaining study assessments until study completion as long as such continued participation will not detrimentally affect the health, safety, and welfare of the subject per investigator determination.

[d] Once subject/caregiver competence is documented, the subject/caregiver administers study drug daily.

[e] Must be completed prior to administration of the first dose of study drug in Part 2. Additional observers identified after Screening must be trained.

[f] PKU history includes highest blood Phe (and age).

[g] Subjects with documented negative results within the last 4 weeks do not need to be retested.

[h] Sample collection should be performed where allowed by country-specific regulations and EC/IRB.

[i] A full PE will be performed at Screening, Baseline, Week 73, and the SC/ET visit. A brief PE will be performed at other visits. A full PE will include, at a minimum, general appearance (head, eyes, ears, nose, and throat), and an assessment of cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, musculoskeletal, and neurologic systems. A brief PE may be performed at the discretion of the investigator based on the subject's clinical condition and may include, but is not limited to, general appearance and an assessment of cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, musculoskeletal, and neurologic systems.

[j] It is recommended that urine samples are obtained as a first or second morning void. In the event of elevated urinary protein on a test result, a repeat urinalysis should be performed. This repeat urine sample must be performed in the morning at the first or second morning void to allow for accurate test results and may be performed by a home healthcare nurse.

[k] To be done by a local laboratory.

[l] Serum cortisol samples should be taken before study drug administration in the morning before 10:00 am. A home healthcare nurse may collect samples for subject convenience. If 2 results are low and abnormal, the subject will be asked to perform additional sampling for plasma ACTH and a low-dose conventional ACTH stimulation test or the ACTH test method based on the clinical practice at the site.

[m] It is recommended that urine samples be obtained as a first or second morning void. Subjects with a confirmed urine/albumin creatinine ratio of ≥ 100 mg/g should be referred to a nephrologist for consultation if results were within normal range at Baseline. Subjects who had elevated results at Baseline followed by a confirmed subsequent increase of 100-200 mg/g from Baseline should also be referred to a nephrologist for consultation.

[n] To be done locally (if applicable). If urine pregnancy test is positive or equivocal, serum pregnancy test (central laboratory) must be done.

[o] Subjects should record at home all food, beverages, special low-protein foods, and medical foods consumed during the 3 consecutive days preceding a study visit. No change in protein (dietary and medical food) intake is allowed in Part 1. During Screening/Run-in, 3-day diet diary assessments are performed 3 times, each time covering the 3-day periods prior to blood Phe assessments (and collected on the day of those assessments). The 3 Screening/Run-in blood Phe assessments are scheduled to occur (1) at Day -25 to Day -23; (2) 7 to 10 days later (ie, in the Day -18 to Day -13 window); and (3) at Day 1 pre-dose. Thus, the 3 day diet assessments will assess diet for (1) the 3 days within the Day -28 to Day -23 window immediately prior to the first blood Phe assessment; (2) the 3 days within the Day -21 to Day -15 window immediately prior to the second blood Phe assessment; and (3) for Day -3 to Day 1.

[p] Blood is collected for blood Phe analysis after fasting a minimum of 2.5 hours. In addition to the blood draws for blood Phe analysis collected in the clinic and shown in the SoA, there are 3 required blood collections that may be drawn either at the clinic or elsewhere (eg, at home). These are to be collected at Weeks 11, 15, and 19.

[q] Completed by parent/guardian.

[r] Completed by parent/guardian.

[s] Between scheduled clinic visits, the clinic staff will contact the subject weekly to ask about any AEs or concomitant medications, and to answer questions.

[t] AEs and concomitant medications should be noted whenever a subject is assessed by study personnel. Following signed informed consent all AEs and SAEs are collected throughout Part 1 or until the Early Termination Visit, whichever occurs last.

Table 1.3.4: Schedule of Activities for Part 2 (Pegvaliase Treatment for Subjects Randomized to Diet-Only Treatment Arm)

	Study Week	73[b]	74	75	76	77	78	79	80	81	85	89	93	97	101	105	109	113	117	121	125	129	133	137	141	145	149	153		
	Study Day	505	512	519	526	533	540	547	554	561	589	617	645	673	701	729	757	785	813	841	869	897	925	953	981	1009	1037	1065	HRV[c]	SC/ET[d]
Assessment [a]	Window (Days)	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7		
Drug administration training [e]		X																												
Study drug observer training [f]		X																												
12 lead ECG		X																												X
Physical examination [g]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Weight		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Height		X				X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
Vital signs		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Clinical safety laboratory tests [h]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
C-reactive protein		X								X		X		X		X		X		X		X		X		X		X	X	X
Erythrocyte sedimentation rate [i]		X																												X
Serum cortisol [j]		X												X						X						X				X
Complements C3 and C4		X				X				X	X	X	X	X		X		X		X		X		X		X		X	X	X
Tryptase		X																											X	
Urine albumin/creatinine ratio [k]		X								X		X		X		X		X		X		X		X		X		X	X	X
Urine pregnancy test [l]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
3-day diet diary [m]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
PK (plasma pegvaliase) [n]		X				X				X	X	X	X	X		X		X		X		X		X		X		X		X
Blood for exploratory analyses [o]		X				X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
Blood Phe, tyrosine [p]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
Immunogenicity assessments [q]		X	X	X	X	X	X	X	X	X	X	X	X	X		X		X		X		X		X		X		X	X	X
ADHD-RS IV [r]		X									X			X			X			X			X			X				X
BRIEF [s]		X									X			X			X			X			X			X				X
Weekly call (non-visit weeks) [t]																														
Adverse events [u]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

Assessment [a]	Study Week	73[b]	74	75	76	77	78	79	80	81	85	89	93	97	101	105	109	113	117	121	125	129	133	137	141	145	149	153	HRV[c]	SC/ET[d]
	Study Day	505	512	519	526	533	540	547	554	561	589	617	645	673	701	729	757	785	813	841	869	897	925	953	981	1009	1037	1065		
	Window (Days)	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7		
Concomitant medication [u]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Administer study drug [v]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		

ACTH, adrenocorticotropic hormone; ADHD-RS IV, attention deficit hyperactivity disorder rating scale IV; BRIEF, Behavior Rating Inventory of Executive Function; ECG, electrocardiogram; eCRF, electronic Case Report Form; HRV, hypersensitivity reaction visit; NSAID, non-steroidal anti-inflammatory drug; PE, physical examination; PK, pharmacokinetics; PKU, phenylketonuria; SC/ET, Study Completion/Early Termination visit.

[a] Scheduled visits take place at the hospital setting (for all dosing visits for the first 8 weeks, and for all dose escalation visits), clinic, or, with the approval of the investigator or designee, virtually. For virtual visits the investigator/designee performs assessments while online with the subject, apart from specimen collections and vital signs, which are performed by home healthcare personnel. Non-clinic visits are via telephone. Assessments are performed pre-dose unless otherwise specified.

[b] For Cohort B control subjects, the Week 73 visit marks both the end of Part 1, when their PKU is treated with diet alone, and the beginning of Part 2, when they receive pegvaliase. The Week 73 pre-dose assessments constituting the end of Part 1 are shown in the Week 73 column of Table 1.3.3. These same assessments, used as baseline assessments for Part 2, are shown in the Week 73 column of Table 1.3.4, along with the administration of study drug that marks the beginning of Part 2.

[c] If the investigator determines that the NCI-CTCAE Grade ≥ 3 hypersensitivity reaction is related to administration with study drug, the subject will be asked to return to the clinic within 24 hours of event onset for a hypersensitivity reaction visit (HRV). Sampling for anti-pegvaliase IgE should be performed 8 to 24 hours after event onset and before the next dose of study drug.

[d] Subjects should perform the Study Completion (SC) Visit 4 weeks after the last dose of study drug or after completion of study participation, whichever occurs last. For subjects who terminate from the study early, the Early Termination (ET) Visit occurs within 4 weeks of the last study drug dose or after withdrawal from study participation, whichever occurs last. Subjects who discontinue from study drug early will be asked to continue to complete the remaining study assessments until study completion as long as such continued participation will not detrimentally affect the health, safety, and welfare of the subject per investigator determination.

[e] Once subject/caregiver competence is documented, the subject/caregiver administers study drug daily.

[f] Must be completed prior to administration of the first dose of study drug. Additional observers identified after screening must be trained.

[g] A full PE will be performed at Week 73, Week 145, and the SC/ET visit. A brief PE will be performed at other visits. A full physical examination will include, at a minimum, general appearance (head, eyes, ears, nose, and throat), and an assessment of cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, musculoskeletal, and neurologic systems. A brief physical examination may be performed at the discretion of the investigator based on the subject's clinical condition and may include, but is not limited to, general appearance and an assessment of cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, musculoskeletal, and neurologic systems.

[h] It is recommended that urine samples are obtained as a first or second morning void. In the event of elevated urinary protein on a test result, a repeat urinalysis should be performed. This repeat urine sample must be performed in the morning at the first or second morning void to allow for accurate test results and may be performed by a home healthcare nurse.

[i] To be done by a local laboratory.

[j] Serum cortisol samples should be taken before study drug administration in the morning before 10:00 am. A home healthcare nurse may collect samples for subject convenience. If 2 results are low and abnormal, the subject will be asked to perform additional sampling for plasma ACTH and a low-dose conventional ACTH stimulation test or the ACTH test method based on the clinical practice at the site.

[k] It is recommended that urine samples be obtained as a first or second morning void. Subjects with a confirmed urine/albumin creatinine ratio of ≥ 100 mg/g should be referred to a nephrologist for consultation if results were within normal range at Baseline. Subjects who had elevated results at Baseline followed by a confirmed subsequent increase of 100-200 mg/g from Baseline should also be referred to a nephrologist for consultation.

[l] To be done locally (if applicable). If urine pregnancy test is positive or equivocal, serum pregnancy test (central laboratory) must be done.

[m] Subjects should record at home all food, beverages, special low-protein foods, and medical foods consumed during the 3 consecutive days preceding a study visit. No change in protein (dietary and medical food) intake is allowed in Part 1.

[n] Blood is collected before study drug administration (pre-dose) within 2 hours of dosing. Intensive PK sampling will be performed at Week 145 and at dose escalation visits in all subjects. Samples will be taken at pre-dose (within 2 hours of dosing), 2, 4, 8, 12, and 24 hours post dose. The 24-hour sample will be taken prior to the next daily dose.

[o] Sample collection should be performed where allowed by country-specific regulations and EC/IRB.

[p] Blood is collected for blood Phe analysis after fasting a minimum of 2.5 hours. In addition to the blood draws for blood Phe analysis collected in the clinic and shown in the SoA, there are 5 required blood collections that may be drawn either at the clinic or elsewhere (eg, at home). These are to occur at Weeks 83, 87, and 91, and 2 weeks prior to the planned escalations to 40 and 60 mg/day.

[q] Immunogenicity assays include total anti-pegvaliase antibodies (TAb), anti-PAL IgG, anti-PAL IgM, anti-PEG IgM, anti-PEG IgG, and neutralizing antibodies (NAb). At the HRV, only anti-pegvaliase IgE will be assessed.

[r] Completed by parent/guardian.

[s] Completed by parent/guardian.

[t] Between scheduled clinic visits, the clinic staff will contact the subject weekly to monitor if the subject is experiencing problems with drug administration, to ask about any AEs or concomitant medications, and to answer questions.

[u] AEs and concomitant medications should be noted whenever a subject is assessed by study personnel. Beginning with start of study treatment, All AEs and SAEs are collected until 4 weeks after the last study drug dose or the Study Completion Visit/Early Termination visit, whichever occurs last. If there is a severe (Grade 3) injection site reaction, the subject may be referred to a dermatologist for consultation and a skin biopsy (optional; if clinically indicated). If photographs of the severe injection site reaction are taken, they may be collected by the sponsor.

[v] Subjects must be premedicated with an H1 antagonist, and an H2 antagonist, and an antipyretic approximately 2 to 3 hours prior to each dose of study drug until they reach the Maintenance Phase, defined as when subjects achieve blood Phe ≤ 600 µmol/L while they are on a stable dose of pegvaliase. Premedication may be considered during the Maintenance Phase at the investigator's discretion, eg, approximately 2 to 3 hours prior to study drug for 1 week upon reintroduction of study drug, upon resolution of an AE, following any dose interruption of ≥ 4 days, and for any dose increases in the Extension Phase. Subjects may also be premedicated at any other time in the study per investigator discretion. When an NSAID is administered as a premedication, it should be given with food. For non-clinic visits, subjects are asked about study drug administration.

2 INTRODUCTION

Phenylketonuria (PKU, OMIM 261600) is a rare autosomal recessive genetic disorder that is characterized by an inability of the body to break down the amino acid, phenylalanine (Phe), and is caused by mutations in the gene encoding phenylalanine hydroxylase (PAH). PAH catalyzes the conversion of the essential amino acid Phe to tyrosine, and this enzymatic activity is facilitated by tetrahydrobiopterin (BH4). PAH deficiency results in abnormally elevated concentrations of Phe. The estimated incidence of PKU is 1 in 15,000 births in the United States (US) (NIHCDP 2001).

The pathophysiology of PKU is well understood. A substantial body of evidence confirms that elevated Phe is directly toxic to cells in the brain, where it causes inhibition of protein synthesis in humans and animals (Wall 1990), affects the normal morphology of myelinating proteins (Dyer 1996), and leads to arrested or delayed development of dendrites and synapses in the cerebral cortex (Huttenlocher 2000). Neuropathology in PKU has been shown to derive from oxidative stress, impaired neurotransmitter metabolism, impaired protein synthesis and lipid metabolism, and brain energy metabolism alterations (Schuck 2015). The neurotoxicity of elevated Phe is a direct effect of Phe itself (Kaufman 1989). No abnormalities in Phe metabolites are observed in patients with PKU and normal Phe metabolites are toxic only at concentrations that are much higher than those reported in individuals with PKU.

With newborn screening followed by Medical Nutritional Therapy (MNT), the most severe manifestation of the disease, mental retardation, is prevented. Phe restriction with MNT involves consumption of semisynthetic medical foods that provide a source of Phe-free protein, modified low-protein foods, a small amount of natural protein (to provide required amounts of Phe), and other supplements (eg, vitamins and minerals) to correct nutritional deficiencies that can result from Phe restriction. Without highly restricted Phe intake and MNT, individuals with PKU quickly reach and sustain toxic concentrations of blood Phe. Individuals with classic PKU may have a complete absence or profound deficiency of PAH and typically show very high elevations of blood Phe (≥ 1200 µmol/L).

Historically, it was recommended that adherence to the Phe-restricted diet could end after age 6, but later studies have shown that patients who maintained Phe control longer had better outcomes in neuropsychological performance and areas of attention and speed of processing (Cerone 1999; Channon 2007; Rey 1996). A robust body of research suggests that early blood Phe control through dietary management is imperative in PKU populations, and that adult intelligence quotient (IQ) levels are correlated with the duration and vigilance of dietary management in childhood and adolescence (Pietz 1997; Smith 2000; Stemerdink 2000; Waisbren 1999).

To be optimally effective, a PKU diet needs to be started immediately after birth and continued for life. The American College of Medical Genetics and Genomics (ACMG) practice guidelines for PKU recommend life-long management and maintenance of metabolic control as essential to optimal functioning of individuals with PKU, with a goal of maintaining blood Phe concentrations ≤ 360 µmol/L (Vockley 2014). Similar European guidelines for the management of PKU recommend treatment target Phe levels of ≤ 600 µmol/L for patients older than 12 years (van Spronsen 2017).

Compliance with diet is a significant component of disease management, but life-long dietary compliance can be extremely difficult. A study conducted over 6 years at 4 treatment centers showed that dietary compliance in PKU populations was found to decline with age. Specifically, during infancy and in pre-school aged children, compliance with restrictive PKU diet is high because their diet is carefully managed by a parent and nutritionist. However, when PKU children start school, their blood Phe levels increase because of changes in socialization and changes in their palate, making compliance to a Phe-restricted diet more challenging (Walter 2002; Walter 2004). As children age, they become more likely to find formula and synthetic low-Phe foods distasteful and they become exposed to a broader range of food and peer pressure, both of which encourage noncompliance (MacDonald 2010). Adolescents/young adults with PKU are at particular risk for non-compliance with MNT as they transition away from childhood when parental oversight of dietary control decreases (Brown 2016). In addition, adolescents/young adults who have stopped MNT and attempt to resume a Phe-restricted diet into adulthood have difficulty maintaining the diet, with 31% of patients no longer maintaining dietary control after 10 months of restarting (Schuett 1985).

Therefore, despite the best efforts of the medical community and patients, life-long therapy with MNT with Phe restriction is not sustainable nor feasible as a treatment option for the majority of patients with PKU (Walter 2002; Burton 2010; Aoki 2007). The inability to adhere to MNT results in uncontrolled blood Phe levels associated with executive dysfunction, depression, and a variety of behavioral and psychiatric problems (Moyle 2007; Pietz 1997; Smith 2000; Waisbren 1999). Adolescents with PKU with poor metabolic control represent a patient population in great need of additional therapeutic options.

BioMarin has developed pegvaliase as a subcutaneous enzyme substitution treatment to reduce blood Phe levels in adult patients with PKU. Enzyme replacement therapy with PAH is not feasible due to the instability of PAH in plasma and the difficulty with efficiently delivering an intact, active form of the enzyme to the liver (Harding 2003; Kim 2004). Pegvaliase is a genetically modified phenylalanine ammonia lyase (PAL) enzyme product of

the cyanobacterium *Anabaena variabilis*, which is PEGylated to decrease immunogenicity and increase half-life. Unlike PAH, the PAL enzyme is active in plasma and, independently of cofactor BH4 which is required for PAH activity, catalyzes Phe to trans-cinnamic acid (t-CA) and ammonia, which are metabolized in the liver and excreted in the urine, respectively. The pegvaliase mechanism of action corrects the underlying PAH deficiency that leads to inability to metabolize Phe, increased Phe levels in the body, and consequent neurocognitive, neuropsychiatric, and executive functioning deficits.

A comprehensive review of pegvaliase is contained in the Investigator's Brochure (IB). Investigators must review this document prior to initiating this study.

2.1 Study Rationale

Although it is recommended that adolescents with PKU maintain good metabolic control as they move into adulthood (Vockley 2014), in separate surveys > 60% of adolescents (13 to 17 years of age) in the US have blood Phe concentrations above recommended targets (Jurecki 2017) and 79% of patients 15 to 19 years report blood Phe concentrations above targets (Walter 2002). Adolescents aged 12 to 17 years with PKU report difficulties with maintaining blood Phe control as they transition away from childhood when parental oversight of dietary and blood Phe control decreases (Brown 2016; van Spronsen 2017). In a 2015 National PKU Alliance (NPKUA) survey conducted among over 625 adults and families, approximately 25% of those caregivers indicated that their children's average blood Phe levels were in excess of the ACMG-recommended targeted range (120 to 360 µmol/L), and over half of all respondents (adults and caregivers) reported that it was somewhat to very difficult to manage their PKU (Brown 2016).

Adolescent patients report difficulties adhering to a Phe-restricted diet to maintain blood Phe levels within a safe range (Brown 2016; van Spronsen 2017), and a clinic survey assessing compliance with clinical guidance and dietary restriction showed that 60% of adolescents aged 13 to 17 were non-compliant, with an associated increase of Phe levels to > 360 µmol/L in 50% of patients and Phe levels > 600 µmol/L in 22% of patients. The same survey demonstrated that non-compliance starts as early as ages 5 to 12, and non-adherence to guidelines increases with age.

For adolescent patients at increased risk for loss of metabolic control maintained during childhood, a variety of adverse neuropsychological deficits (including deficits in executive functioning and psychiatric symptoms, such as anxiety, depression, and phobias) develop later in life when there is relaxation of blood Phe control (Huijbregts 2018; Diamond 1997). Adolescent PKU patients with blood Phe levels ≤360 µmol/L have been shown to perform better at neuropsychological and neurocognitive tasks (Huijbregts 2002; Jahja 2014).

The approval of Palynziq addressed a previous unmet medical need, providing adult PKU patients with a treatment that substantially reduced blood Phe concentrations and resulted in the stabilization of Phe above and beyond diet. The reduction and stabilization of blood Phe were associated with improvement in unmanaged symptoms, including neurocognitive symptoms. There is potential for improvement in these outcomes for adolescents as well given the demonstrated efficacy in adults. Therefore, expansion of Palynziq use to include adolescent patients 12 to 17 years with uncontrolled blood Phe concentrations greater than 600 µmol/L on existing management would broaden the patient population who can benefit from Palynziq treatment and address a currently underserved patient population.

The pathogenesis and pathophysiology of PKU, driven by an absence or deficiency in PAH enzyme leading to high Phe concentration, is applicable to both adolescents and adults. The pegvaliase mechanism of action to correct the underlying metabolic deficiency of PAH gene is the same in both adults and adolescents. Adolescent PKU patients are not expected to have specific ongoing developmental changes that could result in a different safety or efficacy outcome with pegvaliase treatment.

2.2 Background

Palynziq received marketing authorization in the US and European Union (EU). The original Biologics License Application (BLA) for Palynziq was approved by the Food and Drug Administration (FDA) at dosages up to 40 mg/day on 24 May 2018, to reduce blood Phe concentrations in adult patients with phenylketonuria (PKU) who have uncontrolled blood Phe concentrations greater than 600 µmol/L on existing management (BLA STN 761079). A supplemental Biologics License Application (sBLA) that included the addition of the maximum maintenance dose of 60 mg/day and long-term safety and efficacy data for adults was approved by the FDA on 06 October 2020. A Risk Evaluation and Mitigation Strategy (REMS) is a required element of the Palynziq approval in the US. Palynziq was approved by the European Commission on 03 May 2019 at dosages up to 60 mg/day to reduce blood Phe concentrations in patients aged 16 years and older who have inadequate blood Phe control (defined as blood Phe levels greater than 600 µmol/L) despite prior management with available treatment options.

BioMarin recognizes that PKU is a serious genetic disease diagnosed at birth that requires strict management of blood Phe levels from birth and continuing through life. Now that a positive benefit to risk profile has been established in adults, BioMarin is proposing this pegvaliase clinical study in adolescent PKU patients (ages 12 to 17 years old (US), inclusive, and 12 to 15 years old (EU), inclusive).

2.3 Benefit/Risk Assessment

Regulatory approval of Palynziq in the US and the EU was based on demonstration of efficacy from the Phase 2 and Phase 3 data of the pegvaliase clinical development program. The approval of pegvaliase addressed a previous unmet medical need, providing adult PKU patients with a treatment that substantially reduced blood Phe concentrations and resulted in the stabilization of Phe above and beyond diet. This reduction and stabilization of blood Phe was also associated with improvement in unmanaged symptoms including neurocognitive symptoms. Given the demonstrated efficacy in adults, there is the potential for improvement in these outcomes for adolescents.

The important identified risks for pegvaliase include acute systemic hypersensitivity reaction (equivalent to anaphylaxis for the purpose of this protocol; see Section 8.3.8.1 for how this risk is defined), angioedema, serum sickness, hypophenylalaninemia, persistent arthralgia, and severe injection site reaction (see Section 8.3.8 for event definitions). These events have been effectively treated and managed in the clinical trial and post-marketing settings. Healthcare providers, patients, and observers are trained in the recognition and management of both an acute systemic hypersensitivity reaction or angioedema, including the use of injectable epinephrine (adrenaline) and the need to seek immediate medical assistance. Premedication is required during Induction/Titration Dosing to help mitigate the risk of hypersensitivity reactions, including acute systemic hypersensitivity reactions. While hypophenylalaninemia has not been reported frequently in the post-marketing setting, it was observed during the clinical studies, where it was managed with decreases in pegvaliase dosing and increases in dietary protein intake. Subjects will undergo frequent blood Phe monitoring during induction and titration and throughout dosing and will be required to dose reduce with confirmed hypophenylalaninemia (ie, 2 consecutive blood Phe levels < 30 µmol/L).

The important potential risks of treatment with pegvaliase also include potential complications of immune complex formation resulting in end-organ damage, fetal developmental toxicity, and acute systemic hypersensitivity reaction in patients taking other concurrent injectables containing PEG. No evidence of immune complex-mediated end organ damage or any early clinical signs potentially related to immune complex mediated disease have been reported in the clinical trial or commercial setting. Concurrent injectables containing PEG are prohibited during study treatment with the exception of COVID vaccinations (see Section 6.8.1).

The potential risks are justified by the anticipated benefits that may be afforded to adolescent subjects. Each subject will have a comprehensive plan that includes frequent monitoring of

adverse events, blood Phe levels, and pregnancy during the study. Safety will be assessed by adverse event reporting and clinical laboratory assessments.

More detailed information about the known and expected benefits and risks and reasonably expected adverse events of pegvaliase may be found in the IB.

2.3.1 Risk Assessment

Table 2.3.1.1: Risks Associated with Study Drug

Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Acute systemic hypersensitivity reaction	In the completed Phase 2 and Phase 3 clinical trials, 16 out of 285 (6%) subjects experienced 25 episodes of acute systemic hypersensitivity reactions of any severity. Acute systemic hypersensitivity reactions were managed by administration of adrenaline (44%; 11/25 episodes), corticosteroids (56%; 14/25 episodes), antihistamines (56%; 14/25 episodes), and/or oxygen (8%; 2/25 episodes). Four out of 16 (1%; 4/285) subjects experienced a total of 5 episodes of an acute systemic hypersensitivity reaction that were considered severe. The underlying mechanism for acute systemic hypersensitivity reaction observed in clinical trials was non-IgE mediated Type III (immune-complex mediated) hypersensitivity. Acute systemic hypersensitivity reaction was most frequent during the Induction/Titration Phase (5% of subjects; 19 episodes over mean treatment duration of 13 months) and decreased in the Maintenance Phase (2% of subjects; 6 episodes over a mean treatment duration of 33 months). The risk of an acute systemic hypersensitivity reaction occurring is 6-fold higher in the Induction/Titration Phase compared to the Maintenance Phase.	To mitigate the risk of an acute systemic hypersensitivity reaction, an adult observer is required for a minimum of 1 hour post injection and premedication including H1 and H2 antagonists and an antipyretic is required 2 to 3 hours before each injection until subjects reach the Maintenance Phase.[a] Premedication may be considered during the Maintenance Phase at the investigator's discretion. Subjects will be provided with 2 epinephrine injectors and instructed to carry both injectors with them at all times. Subjects and adult observers will be trained to recognize the signs and symptoms of anaphylaxis and, if these reactions occur, to call for emergency medical support and administer an epinephrine injector. For the duration of the study, each subject will be contacted weekly by study site personnel to monitor for drug administration problems and adverse events (AEs). Risk mitigation measures also include dosing instructions for other hypersensitivity adverse events (Table 6.5.1.1).
Angioedema	In the completed Phase 2 and Phase 3 clinical trials, 21 out of 285 (7%) subjects experienced 37 episodes of mild to moderate episodes of angioedema (symptoms include one or more of the following: pharyngeal edema, swollen tongue, lip swelling, mouth swelling, eyelid edema and face edema) occurring independent of acute systemic	To mitigate the risk of hypersensitivity reactions, premedication including H1 and H2 antagonists and an antipyretic is required 2 to 3 hours before each injection until subjects reach the Maintenance Phase.[a] Premedication may be considered during the maintenance phase at the investigator's discretion.

Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
	hypersensitivity reaction. Angioedema was most frequent during the Induction/Titration Phase (6% of subjects; 27 episodes over mean treatment duration of 13 months) and decreased in the Maintenance Phase (3% of subjects; 10 episodes over mean treatment duration of 33 months). The risk of angioedema occurring is 4.5-fold higher in the Induction/Titration Phase compared to the Maintenance Phase.	An adult observer is required for a minimum of 1 hour post injection throughout the study. For the duration of the study, each subject will be contacted weekly by study site personnel to monitor for drug administration problems and AEs. Risk mitigation measures also include dosing instructions for hypersensitivity adverse events (Table 6.5.1.1).
Serum sickness	In the completed Phase 2 and Phase 3 clinical trials, serum sickness was reported in 7 out of 285 (2%) subjects. Serum sickness was most frequent during the Induction/Titration Phase (2% of subjects; 6 episodes over mean treatment duration of 13 months) and decreased in the Maintenance Phase (0.6% of subjects; 1 episode over mean treatment duration of 33 months). The risk of serum sickness occurring is greater than 2-fold higher in the Induction/Titration Phase compared to the Maintenance Phase.	To mitigate the risk of hypersensitivity reactions, premedication including H1 and H2 antagonists and an antipyretic is required 2 to 3 hours before each injection until subjects reach the Maintenance Phase of dosing.[a] Premedication may be considered during the Maintenance Phase at the investigator's discretion. An adult observer is required for a minimum of 1 hour post injection throughout the study. For the duration of the study, each subject will be contacted weekly by study site personnel to monitor for drug administration problems and AEs. Risk mitigation measures also include dosing instructions for hypersensitivity adverse events (Table 6.5.1.1).
Persistent arthralgia (> 6 month duration)	In the completed Phase 2 and Phase 3 clinical trials, 245 out of 285 (86%) subjects experienced episodes consistent with arthralgia (including back pain, musculoskeletal pain, pain in extremity, and neck pain). Arthralgia occurred as early as the first dose and can occur at any time during treatment. Arthralgia was most frequent during the Induction/Titration Phase (79% of subjects; 1269 episodes over mean treatment duration of 13 months) and decreased in the Maintenance Phase (67% of subjects; 673 episodes over mean treatment duration of 33 months). The risk of arthralgia occurring is 3.1-fold higher in the Induction/Titration Phase compared to the Maintenance Phase. Seven (7%) percent of the subjects experienced persistent arthralgia (≥ 6 months). The risk of persistent	To mitigate the risk of hypersensitivity reactions, premedication including H1 and H2 antagonists and an antipyretic is required 2 to 3 hours before each injection until subjects reach the Maintenance Phase of dosing.[a] Premedication may be considered during the Maintenance Phase at the investigator's discretion. An adult observer is required for a minimum of 1 hour post injection throughout the study. For the duration of the study, each subject will be contacted weekly by study site personnel to monitor for drug administration problems and AEs. Risk mitigation measures also include dosing instructions for hypersensitivity adverse events (Table 6.5.1.1).

Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
	arthralgia may have significant impact on quality of life. Severe arthralgia (severe pain limiting self-care activities of daily living) was experienced in 5% of subjects. Dose was not changed for 96% of episodes and all persistent arthralgia episodes resolved without sequelae.	
Severe injection site reaction	Injection site reaction (ISR) was reported in 266 out of 285 (93%) subjects. The most common ISR (occurring in at least 10% of subjects) was reaction, erythema, bruising, pruritus, pain, swelling, rash, induration, and urticaria. Injection site reaction was most frequent during the Induction/Titration Phase (90% of subjects; 3908 episodes over mean treatment duration of 13 months) and decreased in the Maintenance Phase (66% of subjects; 1226 episodes over mean treatment duration of 33 months). The risk of an ISR occurring is 5.2-fold higher in the Induction/Titration Phase compared to the Maintenance Phase. Three ISRs consistent with granulomatous skin lesions were reported (each reaction occurring in 1 patient): granulomatous dermatitis (occurred 15 months after pegvaliase treatment and lasted 16 days), xanthogranuloma (occurred 12 months after pegvaliase treatment and lasted 21 months), and necrobiosis lipoidica diabeticorum (occurred 9 months after pegvaliase treatment and lasted 9 months). Necrobiosis lipoidica diabeticorum was treated with steroid injections and was complicated by a *Pseudomonas* infection. All of these injection site reactions resolved.	To mitigate the risk of hypersensitivity reactions, premedication including H1 and H2 antagonists and an antipyretic is required 2 to 3 hours before each injection until subjects reach the Maintenance Phase of dosing.[a] Premedication may be considered during the Maintenance Phase at the investigator's discretion. An adult observer is required for a minimum of 1 hour post injection throughout the study. For the duration of the study, each subject will be contacted weekly by study site personnel to monitor for drug administration problems and AEs. Risk mitigation measures also include dosing instructions for hypersensitivity adverse events (Table 6.5.1.1). Any subject who experiences a severe (Grade 3) skin reaction will be asked to consult with a dermatologist for diagnosis and, if clinically indicated, a skin biopsy will be recommended. A Skin Reaction eCRF must be completed.
Hypophenylalaninemia	In the completed Phase 2 and Phase 3 clinical trials, 46% of the subjects developed hypophenylalaninemia (blood phenylalanine levels below 30 µmol/L on two consecutive measurements). The risk of hypophenylalaninemia occurring is 2.1-fold higher in the maintenance phase compared to the Induction/Titration phase. In the completed adult clinical studies, dose reductions were effective in	Blood Phe levels will be frequently monitored during induction and titration dosing and regularly monitored during maintenance dosing in both the Primary Treatment Phase and the Extension Phase of the study. Risk mitigation measures, including protocol-required adjustments in the pegvaliase dose and/or dietary intake of Phe, will be implemented to mitigate the potential risk of chronic

Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
	managing hypophenylalaninemia in subjects experiencing hypophenylalaninemia despite appropriate levels of protein intake.	hypophenylalaninemia levels (< 30 µmol/L) in adolescent subjects.
Complications of immune complex formation resulting in end-organ damage	No evidence from clinical trials or post-marketing reports of immune complex-mediated end organ damage or any early clinical signs potentially related to immune complex mediated disease.	Risk mitigation measures include monitoring clinical laboratory tests (chemistry, hematology, urinalysis) and adverse events for immune complex-mediated end-organ damage. These mitigations will also allow early detection of adverse effects of PEG accumulation. For the duration of the study, each subject will be contacted weekly by study site personnel to monitor for AEs.
Fetal development toxicity	There are limited data from the use of pegvaliase in pregnant women. Animal studies have shown maternal reproductive toxicity that was associated with decreased blood phenylalanine concentrations below normal levels. Uncontrolled blood phenylalanine levels (hyperphenylalaninemia) before and during pregnancy are associated with increased risk for miscarriage, major birth defects (including microcephaly and major cardiac malformations), intrauterine fetal growth retardation and future intellectual disability with low IQ. Additional risk to the unborn child due to hypophenylalaninemia is not established.	If sexually active, male or female subjects must not plan to become pregnant (self or partner) and must use 2 acceptable methods of contraception while participating in the study beginning at Screening and for 4 weeks after discontinuing study drug. Contraceptive use must be consistent with the guidance provided in Appendix 4, Section 10.4 of this protocol.
Unpredictable immune-mediated response in patients < 16 years	The adult development program was restricted to subjects aged > 16 years of age. Review of spontaneous adverse reaction reports in patients < 16 years of age, using Palynziq in the commercial setting has not identified any unpredictable immune responses. The adverse reactions reported in patients < 16 years of age are consistent with the known safety profile of Palynziq as presented in the USPI and SmPC.	To mitigate the risk of hypersensitivity reactions, premedication including H1 and H2 antagonists and an antipyretic is required 2 to 3 hours before each injection until subjects reach the Maintenance Phase. [a] Premedication may be considered during the Maintenance Phase at the investigator's discretion. An adult observer is required for a minimum of 1 hour post injection throughout the study. For the duration of the study, each subject will be contacted weekly by study site personnel to monitor for drug administration problems and AEs. Risk mitigation measures also include dosing instructions for hypersensitivity adverse events (Table 6.5.1.1).

Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Acute systemic hypersensitivity reactions in patients taking other concurrent injectables containing PEG	PEGylated proteins have the potential to elicit an immune response. Because antibodies bind to the PEG portion of pegvaliase, there may be potential for binding with other PEGylated therapeutics and increased hypersensitivity to other PEGylated injectables. In a single dose study of pegvaliase in adult subjects with PKU, two patients receiving concomitant injections of medroxyprogesterone acetate suspension containing PEG experienced hypersensitivity reactions.	Risk mitigation measures include prohibiting any injectable drugs containing polyethylene glycol (PEG; other than pegvaliase), including medroxyprogesterone injection, within 3 months prior to the start of Screening/Run-in and during study participation with the exception of COVID vaccinations (see Section 6.8.1).

[a] Subjects are in the Maintenance Phase when they achieve blood Phe ≤600 µmol/L while they are on a stable dose of pegvaliase. This is distinct from Maintenance Dosing, defined as the period following Induction/Titration Dosing when Maintenance Doses (20, 40, or 60 mg/day) are administered, as outlined in Table 6.5.1.

2.3.2 Benefit Assessment

The overall clinical benefit of pegvaliase was established in patients with PKU based on data from completed Phase 2 and Phase 3 clinical studies. The approval of Palynziq addressed a previous unmet medical need, providing adult PKU patients with a treatment that substantially reduced blood Phe concentrations and resulted in the stabilization of Phe above and beyond diet. This reduction and stabilization was also associated with improvement in unmanaged symptoms including neurocognitive symptoms. Given the demonstrated efficacy in adults, there is the potential for improvement in these outcomes for adolescents.

2.3.3 Overall Benefit/Risk Conclusion

Results from the robust pegvaliase clinical development program demonstrated that pegvaliase treatment up to 60 mg/day demonstrated a positive benefit-risk profile in patients with PKU with uncontrolled blood Phe levels > 600 µmol/L.

Taking into account the measures taken to minimize risk to subjects participating in this study, the anticipated benefits that may be afforded to adolescent PKU patients treated with pegvaliase outweigh the potential risks.

3 OBJECTIVES AND ENDPOINTS

The primary, secondary, and exploratory objectives and associated endpoints are shown in Table 3.1.

Table 3.1: Objectives and Endpoints

Objectives	Endpoints
Primary	
To evaluate the efficacy of pegvaliase in adolescent subjects with PKU	The primary efficacy endpoint is change in blood Phe concentration from treatment naïve baseline following 72 weeks on study. Change in blood Phe from baseline is compared between subjects in the active (pegvaliase) and the control (diet-only) treatment arms (see Section 9.4.2).
To evaluate the safety of pegvaliase in adolescent subjects with PKU	• Adverse events (AEs), including serious AEs (SAEs) • Clinical laboratory tests (chemistry, hematology, and urinalysis) results • Vital signs • Physical examination (including height, weight) • Electrocardiogram (ECG) test results • Immunogenicity test results (anti-PEG IgG, anti-PEG IgM, anti-PAL IgG, anti-PAL IgM, TAb, NAb, and anti-pegvaliase IgE [hypersensitivity reaction visits only]).
Secondary	
To characterize total dietary protein intake in adolescent subjects with PKU after pegvaliase treatment	Total dietary protein intake must remain stable for change in blood Phe, the primary endpoint, to be accurately assessed. To gauge the stability of total protein intake, the change from baseline at Week 73 in total dietary protein intake (defined as the sum of the protein intake from medical food and intact food) will be measured (see Section 9.4.3).
To characterize the pharmacokinetics (PK) of pegvaliase in adolescent subjects with PKU	Trough plasma PK samples will be collected and analyzed as specified in the SoA. PK parameters assessed with intensive PK sampling will include $AUC_{0\text{-}24hr,ss}$, $C_{max,ss}$, $T_{max,ss}$, $t_{1/2}$, $CL_{ss/F}$, and $V_{dz/F}$. For subjects randomized to the active (pegvaliase) treatment arm, intensive PK sampling will be performed at Week 73 and for dose escalations to 20, 40, and 60 mg/day. For subjects randomized to the control (diet-only) treatment arm, there are no PK assessments in Part 1. In Part 2, intensive PK sampling will be performed at Week 145 and for dose escalations to 20, 40, and 60 mg/day (see Section 8.4).

Objectives	Endpoints
Exploratory Objectives	
To evaluate the effect of pegvaliase treatment on neurocognitive outcomes in adolescent subjects with PKU.	• The ADHD Rating Scale IV inattention subscale (ADHD-RS IV) rated by the parent/guardian and performed at Baseline (Day 1, pre-dose) and every 12 weeks throughout the study (see Section 8.1.3.1.1). • The Behavior Rating Inventory of Executive Function (BRIEF) rated by the parent/guardian and performed at Baseline (Day 1, pre-dose) and every 12 weeks throughout the study (see Section 8.1.3.1.2).
To compare subjects' optimal maintenance pegvaliase dose across different age groups within the study	• Time to subjects' optimal maintenance dose compared to older age subsets • Number of subjects who require the highest (60 mg/day) maintenance dose • Percentage/number of subjects in the blood Phe target ranges of < 600 µmol/L and < 360 µmol/L.
Explore the biochemical, molecular, and cellular aspects of PKU.	Blood will be collected to evaluate biochemical, molecular, and cellular aspects of PKU and to develop the assays used for these evaluations.
PAH genotyping	Testing will be performed on samples collected Day 1 pre-dose where allowed by local health authorities and the EC/IRB.

4 STUDY DESIGN

4.1 Overall Design

This Phase 3 multi-center study is designed to evaluate the safety and efficacy of pegvaliase administered daily to adolescent subjects (ages 12 to 17 years old (US), inclusive, and 12 to 15 years old (EU), inclusive) with PKU. Pegvaliase will be subcutaneously injected in a hospital setting for the first 8 weeks and thereafter at the clinic or at home by a trained subject, caregiver, adult observer, or visiting healthcare provider. Pegvaliase will also be injected in a hospital setting for any subsequent dose escalations. The study design is summarized in Figure 1.2.1.

The study will enroll approximately 54 US and EU subjects (ages 12 to 17 years old (US), inclusive, and 12 to 15 years old (EU), inclusive) who will be evaluated using an open-label, 2-arm, randomized control design, with diet-only management of PKU as the control. Subjects will be randomized in a 2:1 ratio to the active and control arms, respectively, with 36 subjects receiving pegvaliase and 18 subjects managing their PKU with diet alone.

The primary efficacy endpoint is the change from baseline in blood Phe following 72 weeks on study (the end of Part 1). Beginning Week 73, subjects in the active treatment arm will continue to receive pegvaliase in the Extension Phase (Part 2) for up to an additional 80 weeks (through Week 153) in order to evaluate the long-term safety and efficacy of pegvaliase in adolescent subjects with PKU. Subjects in the diet-only control arm will initiate pegvaliase treatment beginning Week 73 and, from Weeks 73 through 145, follow the same dosing and assessment schedule that the active subjects followed from Weeks 1 through 73, after which they will receive pegvaliase for up to 8 additional weeks (through Week 153).

Eligible subjects are required to have 2 blood Phe concentration measurements > 600 µmol/L during the Screening/Run-in Period (7 to 10 days apart) and an average blood Phe concentration > 600 µmol/L over the past 12 months prior to Screening based on available historical data. Once enrolled, patients will be dosed with pegvaliase according to the I/T/M dosing regimen based on the US package insert (USPI), with the guidance to increase dose depending on individual blood Phe lowering efficacy (per investigator's discretion). Dose should be increased to 40 mg/day if blood Phe is > 360 µmol/L after 24 weeks dosing at 20 mg/day, and increased to 60 mg/day if blood Phe is > 360 µmol/L after 16 weeks dosing at 40 mg/day. If a subject's blood Phe is > 600 µmol/L after 16 weeks of continuous treatment with pegvaliase at 60 mg/day, pegvaliase should be permanently discontinued. BioMarin recommends that the target blood Phe levels should be based on individual blood Phe lowering efficacy.

In Part 1 (through the Week 73 visit), randomized subjects in both the control (diet-only) and the active (pegvaliase) treatment arms will be required to maintain the same level of dietary protein intake they established during Screening/Run-in. In Part 2, subjects in the control (diet-only) arm must continue to maintain this prescribed level of protein intake, whereas subjects randomized to the active (pegvaliase) arm whose blood Phe concentration is ≤ 360 µmol/L for a minimum of 4 weeks may adjust dietary protein intake based on individual subject response to pegvaliase and guidance from the investigator.

4.2 Scientific Rationale for Study Design

This Phase 3 multi-center study is designed to evaluate the safety and efficacy of pegvaliase administered daily to adolescent subjects with PKU. Approximately 54 US and EU subjects (ages 12 to 17 years old (US), inclusive, and 12 to 15 years old (EU), inclusive) will be evaluated using an open-label, 2-arm, randomized control design, with diet-only management of PKU as the control.

A RCT design, with diet-only management of PKU as the control, will allow for robust data collection and minimize the risk of confounding factors influencing the results. An open-label diet-only control arm is the best comparator for this age group as daily placebo injections would place undue burden on adolescent PKU patients.

Except for subjects randomized to pegvaliase in Part 2 whose blood Phe concentration is ≤ 360 µmol/L for a minimum of 4 weeks, subjects in both the control (diet-only) and active (pegvaliase) treatment arms are required to maintain a consistent level of protein intake throughout the study (see Section 4.1 and Section 8.1.2). Subjects are required to maintain a consistent level of protein intake to ensure that the safety and efficacy endpoints are attributable to pegvaliase rather than to changes in dietary protein intake.

Pegvaliase will be subcutaneously injected in a hospital setting for the first 8 weeks and for all subsequent dose escalations, and afterwards at the clinic or at home by a trained subject, caregiver, adult observer, or visiting healthcare provider. Administration at home makes daily treatment less burdensome for subjects, thereby improving treatment compliance.

An adult observer is required for a minimum of 1 hour post injection throughout the study. An adult observer is defined as someone who will be present with the subject during and after pegvaliase administration, is able to recognize the signs and symptoms of an anaphylaxis episode, and is able to call for emergency medical support and administer epinephrine, if warranted. The requirement for an adult observer was added to ensure that subjects self-administering pegvaliase would have help in recognizing and quickly responding to signs and symptoms of anaphylaxis should they emerge.

The primary efficacy endpoint is the change from baseline in blood Phe following 72 weeks on study (the end of Part 1). Blood Phe level is the established clinical marker used to diagnose PKU and assess disease control, and reduction of blood Phe concentration to target levels is the universally acknowledged treatment goal for PKU patients. Published literature support the clinical benefit of lowering blood Phe concentration to ≤ 600 µmol/L for associated improvement in clinical outcomes including cognitive performance, behavior and mood (Palermo 2017; Romani 2017; Burgard 1999; Azen 1996; Smith 1989; Fitzgerald 2000; Costello 1994; Fonnesbeck 2013; Gassio 2003).

In addition, evidence has shown improved clinical benefit with even more aggressive blood Phe lowering targets. In children and adolescents with PKU, studies show that patients with blood Phe levels < 360 µmol/L perform better at neuropsychological and neurocognitive tasks (Huijbregts 2002; Jahja 2014). The findings from these studies demonstrate the need to adequately control blood Phe levels in PKU pediatric patients to below the 600 µmol/L threshold.

4.2.1 Subject Input into Design

BioMarin sought input from the PKU community in designing this study. Parents and a scientist from the National PKU Alliance reviewed portions of the protocol and provided feedback. Among other important input, the community stressed the importance of:

- Setting expectations for study participation, something that is critical to parents during the informed consent process
- Ongoing support from site personnel during the study
- Training for adult observers
- Simplifying, as much as possible, the diet tracking requirements given the time commitment required on the part of families
- Flexibility in scheduling clinic visits and including home and virtual visits where possible

BioMarin has taken this PKU community feedback into account when designing the study:

- Detailed training will be provided for subjects, parents, and adult observers, and will continue throughout the study.
- Diet diaries will be in paper format and, consistent with past studies, diet tracking is restricted to the 3 days prior to study visits.

- Scheduled visits will take place at the clinic or, with the approval of the investigator or designee, virtually. For virtual visits the investigator/designee performs assessments while online with the subject, apart from specimen collections and vital signs, which are performed by home healthcare personnel.

4.3 Justification for Dose and Dose Regimen

Pegvaliase is a daily treatment for patients with PKU that will be administered in an Induction/Titration/Maintenance (I/T/M) regimen described in Section 6.5. Previous clinical studies have suggested the primary clearance mechanism of pegvaliase from plasma is via formation of circulating immune complexes leading to complement activation and removal of the drug by phagocytosis, indicating pegvaliase plasma exposure was driven by immune response. Considering that the immune system matures prior to adolescence (Olin 2018; Georgountzou 2017), the immune response to pegvaliase among adolescent subjects is expected to be similar to the responses observed in 16 to 57-year-old study subjects in the pegvaliase clinical development program.

Following dosing in a hospital setting for the initial doses (first 8 weeks) and apart from subsequent dose escalations to 20, 40, and 60 mg/day, which will also be administered in a hospital setting, pegvaliase will typically be administered at home by the trained subject, though pegvaliase may be also administered at home by a trained caregiver or visiting healthcare provider. In addition to training in drug administration prior to the first in-clinic dose, subjects, caregivers, and adult observers will receive extensive training on how to monitor and respond to possible AEs (including acute systemic hypersensitivity reaction [anaphylaxis]) that may be associated with study drug treatment.

Pegvaliase will be administered using an I/T/M dosing regimen similar to the regimen used in Phase 3 studies performed in the US (165-301 and 165-302) and included in the USPI and SmPC (see Table 6.5.1 and Figure 1.2.2). During pegvaliase clinical development, this dosing regimen was found to help mitigate the onset and severity of hypersensitivity reactions while substantially reducing blood Phe concentrations.

The pegvaliase dose levels in this study have been evaluated in 6 multiple-dose Phase 2 and Phase 3 studies. The dose level for induction in 165-306 will be 2.5 mg once weekly and was chosen because this dose with a once weekly dosing frequency administered during the initial 4 weeks of treatment (ie, induction) used in Studies PAL-002, 165-205, and 165-301 has been associated with a lower incidence and severity of hypersensitivity reactions when compared with 5 days per week dosing frequency at a higher starting dose (0.4 mg/kg) studied in Study PAL-004.

4.4 End of Study Definition

A subject is considered to have completed the study if he/she has completed all scheduled visits, including procedures scheduled at the Study Completion/Early Termination (SC/ET) visit, as shown in the SoA (see Section 1.3), or the study is closed by the sponsor. The end of the study is defined as the date of the last visit of the last subject in the study.

5 STUDY POPULATION

Prospective approval of protocol deviations to eligibility criteria, also known as protocol exemptions, is not permitted. Rescreening is allowed for out of range laboratory values.

5.1 Inclusion Criteria

Subjects are eligible to be included in the study only if all of the following criteria apply:

Age

1. Is 12 to 17 years old (US), inclusive, or 12 to 15 years (EU), inclusive, at the start of the Screening/Run-in Period (Day -28).

Type of Participant and Disease Characteristics

2. Diagnosis of PKU and failure to maintain recommended blood Phe levels on existing management (sapropterin dihydrochloride and Phe-restricted diet) demonstrated by 2 blood Phe concentration measurements > 600 µmol/L during the Screening/Run-in Period (7 to 10 days in between blood Phe assessments) and average blood Phe concentration > 600 µmol/L over the past 12 months (per available data).
3. Willing and able to maintain and adjust dietary and medical protein food intake according to the study protocol under the supervision of a study dietician or adequately trained designee per investigator discretion during study participation.
4. If on medication for ADHD, depression, or other psychiatric disorder, stable dose of medication for ≥ 8 weeks prior to enrollment and willing to maintain stable dose unless a change is medically indicated.
5. An adult (≥ 18 years of age) has been identified who is willing and competent to observe the subject during study drug administration and for a minimum of 1 hour following administration.
6. Able and willing to comply with all study procedures.
7. Generally in good health, as evidenced by physical examination, clinical laboratory evaluations (hematology, chemistry, and urinalysis), and electrocardiogram (ECG) tests performed at Screening.

Sex and Contraceptive/Barrier Requirement

8. Males and females are eligible to participate in this clinical study.
9. Females with childbearing potential must have a negative pregnancy test at Screening/Run-in and be willing to have additional pregnancy tests during the study.
10. If sexually active, male or female subjects must not plan to become pregnant (self or partner) and must use 2 acceptable methods of contraception while participating in the study beginning at Screening and for 4 weeks after discontinuing study drug.

Contraceptive use must be consistent with the guidance provided in Appendix 4, Section 10.4 of this protocol. Acceptable methods of contraception may include:

- Primary forms: hormonal (combination hormone-containing pills, patch, vaginal ring, or intrauterine device) or non-hormonal (copper-containing intrauterine device).
- Secondary forms: barrier forms and other forms of birth control and must include spermicide (eg, male condom; female condom is not an acceptable secondary form).

11. Contraceptive use should be consistent with local regulations regarding the methods of contraception for those participating in clinical studies.

Informed Consent

12. Subjects must be capable of giving signed informed consent as described in Appendix 1, Section 10.1, which includes compliance with the requirements and restrictions listed in the informed consent form (ICF) and in this protocol. In the case of subjects under the age defined by regional law or regulation, parent(s) or guardian(s) must be willing and able to provide written, signed informed consent after the nature of the study has been explained and prior to performance of any research-related procedure. Subjects under the age of majority must be willing and able to provide written assent (if required by local regulations or the IRB/IEC) after the nature of the study has been explained and prior to performance of any research-related procedure.

5.2 Exclusion Criteria

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

Medical Conditions:

1. Concurrent disease or condition that would interfere with study participation or safety (eg, history or presence of clinically significant cardiovascular, pulmonary, hepatic, renal, hematologic, gastrointestinal, endocrine, immunologic, dermatologic, neurological, oncologic, or psychiatric disease).

Prior/Concomitant Therapies:

2. Taking any of the prohibited medications outlined in Section 6.8.1.
3. Previous treatment with pegvaliase.
4. Use of any medication that is intended to treat PKU, including the use of large neutral amino acids, within 14 days prior to the administration of study drug on Day 1.
5. Use or planned use of any injectable drugs containing polyethylene glycol (PEG; other than pegvaliase), including medroxyprogesterone injection, within 3 months prior to the start of Screening/Run-in and during study participation with the exception of COVID-19 vaccinations (Section 6.8.1).

6. A history of organ transplantation or on chronic immunosuppressive therapy.

Prior/Concurrent Clinical Study Experience

7. Use of any investigational product or investigational medical device within 30 days prior to Screening/Run-in or requirement for any investigational agent prior to completion of all scheduled study assessments.

Diagnostic Assessments

8. A positive test for HIV antibody, hepatitis B surface antigen, or hepatitis C antibody.
9. Alanine aminotransferase (ALT) concentration > 2 × the upper limit of normal (ULN).
10. Creatinine > 1.5 × ULN.

Other Exclusions

11. A history of substance abuse (as defined by the American Psychiatric Association: Diagnostic and Statistical Manual of Mental Disorders [DSM]) in the past 12 months or current alcohol or drug abuse.
12. Pregnant or breastfeeding or planning to become pregnant (self or partner) or breastfeeding at any time during the study.
13. Major surgery planned during the study period.
14. Inability to identify and/or communicate to others that the subject is experiencing symptoms of potential anaphylaxis due to cognitive impairment or other reasons.
15. Unwillingness or inability of subject/caregiver to carry auto-injectable epinephrine with them at all times.
16. Any condition that, in the view of the investigator, places the subject at high risk of poor treatment compliance or terminating early from the study.

5.3 Lifestyle Considerations

5.3.1 Meals and Dietary Restrictions

Details of all PKU-specific dietary therapy (eg, Phe intake, amino-acid supplementation) must be recorded in the source document and electronic case report form (eCRF).

Dietary Instruction

Subjects must maintain dietary Phe intake levels that are consistent with their baseline levels for the duration of the study unless clinically indicated, per dietician or adequately trained designee per investigator/designee discretion to support individual nutritional needs during study participation (refer to Section 8.1.2).

Fasting Requirements

Fasting (water only) is required for at least 2.5 hours prior to any blood Phe measurement.

Any time fasting is required for visit assessments, the fasting assessments should be completed and fasting stopped before clinical outcome assessments are performed. The fast can be broken with a snack or meal as preferred.

5.3.2 Activity Restrictions

Subjects should abstain from strenuous exercise for 48 hours before each blood collection for clinical laboratory tests. Subjects may participate in light recreational activities (such as watching television or reading) prior to study visits, as appropriate.

5.4 Screen Failures

Screen failures are defined as subjects who assent to participate (and whose parents/guardians consent to their participation) in the clinical study but who are not subsequently enrolled. A minimal set of screen failure information is required to ensure transparent reporting of screen failure subjects to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and the occurrence of any serious adverse event (SAE).

Individuals who do not meet the criteria for participation in the study may be rescreened. Rescreened subjects should be assigned the same subject number as for the initial screening. If eligibility criteria are met on retest within the screening window, the subject may be enrolled after confirmation by the medical monitor. Subjects who do not meet eligibility criteria within the screening window, will have to perform all Screening Visit procedures and assessments when re-screened.

5.5 Study Stopping Criteria

The independent Data Monitoring Committee (DMC) will be responsible for performing a benefit to risk assessment of pegvaliase in this patient population on a regular (quarterly) and ad hoc basis during the study and for making recommendations to BioMarin with regards to study conduct. The DMC Chair will be informed of all subjects meeting the Individual Stopping Criteria Evaluation for Adverse Events During Pegvaliase Treatment (Section 8.3.8.1.1).

6 STUDY TREATMENT

6.1 Study Treatment(s) Administered

Study treatment is defined as any investigational treatment(s), marketed product(s), placebo, or medical device(s) intended to be administered to a study subject according to the study protocol.

The investigational treatment is pegvaliase (recombinant *Anabaena variabilis* phenylalanine ammonia lyase-PEG). For subcutaneous administration, subjects are supplied with prefilled syringes in 3 sizes. Sizes are 2.5 mg (0.5 mL of 5 mg/mL protein concentration), 10 mg (0.5 mL of 20 mg/mL protein concentration), and 20 mg (1.0 mL of 20 mg/mL protein concentration).

The excipients in this drug product are tromethamine, tromethamine-hydrochloride, sodium chloride, trans-cinnamic acid, and water for injection.

Study treatment details are provided in Table 6.1.1.

Table 6.1.1: Study Treatment

Treatment Name	pegvaliase
Type	drug
Dose Formulation	prefilled syringe
Unit Dose Strength	Each PFS is filled to deliver at least 0.5 or 1 mL of formulation containing 2.5, 10, or 20 mg of pegvaliase
Dosage Level(s)	2.5 mg (1x or 2x per week), 10 mg (2x or 4x per week), 20 mg (daily), 40 mg (daily), 60 mg (daily)
Route of Administration	subcutaneous injection
IMP and NIMP	IMP
Sourcing	provided centrally by the sponsor
Packaging and Labeling	Drug product packaging is identified with lot and identification numbers and provided in a box labeled with the study number and identified to be for investigational use.
Current/Former Name(s) or Alias(es)	pegvaliase, BMN 165, rAvPAL-PEG (PEGylated recombinant Anabaena variabilis phenylalanine ammonia lyase), Palynziq® (trade name)

IMP, investigational medical products; NIMP, non-investigational medical products; PFS, pre-filled syringe.

In addition to pegvaliase, treatment in this study includes a diet in which total protein intake (defined as the sum of protein intake from medical food and intact food) must remain stable and consistent with baseline levels. Specifically, to accurately assess the primary endpoint comparison between the active (pegvaliase) and control (diet-only) treatment arms on the

change from baseline in blood Phe concentration following 72 weeks on study, total protein intake must remain stable and consistent with baseline levels for subjects in both treatment arms through Part 1. In Part 2, subjects in the control arm must continue to maintain this prescribed level of protein intake, whereas subjects randomized to pegvaliase whose blood Phe concentration is ≤ 360 µmol/L for a minimum of 4 weeks may adjust dietary protein intake based on individual subject response to pegvaliase and guidance from the investigator. Details on diet monitoring is provided in Section 8.1.2.

6.1.1 Additional Drug Administration Instructions

Each refilled syringe is intended for a single subcutaneous injection. The recommended injections sites include the front middle of thighs and the abdomen at least 2 inches (5 centimeters) away from the navel. If a caregiver is giving the injection, the top of the buttocks and the back of the upper arms are also appropriate injections sites. The injection sites for administration of pegvaliase should alternate between doses. Pegvaliase must not be injected into moles, scars, birthmarks, bruises, rashes, or areas where the skin is hard, tender, red, damaged, burned, inflamed, or tattooed. If multiple injections are needed for a single dose, injections should be administered at the same time of day and injection sites should be at least 5 cm away from each other. Doses should not be divided over the course of the day.

A subject (or subject-designated caregiver) must meet all of the following criteria to be eligible to administer the drug:

- Has no known cognitive impairments that may increase the safety risk per investigator assessment
- Has no medical history or current medications that may compromise administration of pegvaliase per investigator assessment
- Has completed all required drug administration training and has demonstrated administration competency per investigator assessment, including how to prepare pegvaliase for administration and safely perform the injection

Refer to Section 6.5 for study drug administration requirements regarding the presence of an adult observer and for premedication requirements.

Following a dose interruption of ≥ 4 doses, the investigator should obtain approval from the medical monitor prior to the subject restarting pegvaliase. Dose regimens may be revised provided the investigator obtains approval from the medical monitor prior to starting an alternative dose regimen.

The suitability for self-administration of pegvaliase will be reassessed by the investigator if a subject experiences any issues with self-administration. In addition, if a subject has an

NCI-CTCAE grade 3 or higher AE during the study, the subject's suitability for self-administration of pegvaliase will be reassessed by the investigator.

Between scheduled clinic visits, the clinic staff will contact the subject weekly to monitor if the subject is experiencing problems with drug administration, for any AEs, and to answer questions throughout the duration of the study. The subject must perform the scheduled assessments and procedures in the clinic as outlined in the SoA (Section 1.3). Training materials and other information specific to the study site personnel will be provided in the study reference materials, which includes drug administration training materials and instruction on the following:

- How to prepare and safely perform the injections of pegvaliase
- How to receive, store, and return both used and unused pegvaliase
- How to safely use and dispose of prefilled syringes used for injections of pegvaliase
- How to use a prefilled syringe every time drug is administered
- How to care for the injection site after an injection of pegvaliase
- How to document each pegvaliase injection
- How to identify an AE and how to report this to the study site
- How and when to use epinephrine
- Whom to contact at the study site in case of an emergency

Subjects will be provided with a workbook to document the date and time of pegvaliase injections, injection site, any suspected AEs, and other relevant information. Dosing in response to an AE should be determined by the investigator.

6.2 Preparation/Handling/Storage/Accountability

At the study site, all study drug must be stored at 5 ±3°C (41 ±5°F), under the conditions specified in the IB, and in a secure area accessible only to the designated pharmacists and clinical site personnel. All study drug must be stored and inventoried, and the inventories must be carefully and accurately documented according to applicable state, federal, and local regulations; ICH Good Clinical Practice (GCP); and study procedures. Information regarding storage of study drug in subjects' homes is provided in the study training materials.

The investigator or designee must confirm appropriate temperature conditions have been maintained during transit for all study treatment received and that any discrepancies are reported and resolved before use of the study treatment.

Only subjects enrolled in the study may receive study treatment, and only authorized site staff may supply study treatment. All study treatment must be stored in a secure, environmentally controlled and monitored (manual or automated) area in accordance with the labeled storage conditions, with access limited to the investigator and authorized site staff.

The investigator, institution, qualified pharmacist, or the head of the medical institution (where applicable) is responsible for study treatment accountability, reconciliation, and record maintenance (ie, receipt, reconciliation, and final disposition records).

Subjects are provided instructions for returning used and unused study drug prefilled syringes and for proper disposal at each site visit.

Further guidance and information for the final disposition of unused study treatments are provided in the Pharmacy Manual.

6.3 Measures to Minimize Bias: Randomization and Blinding

This is an open-label study; however, the specific intervention to be taken by a subject will be assigned using an IVRS/IWRS. The site will contact the IVRS/IWRS prior to the start of study intervention administration for each subject. The site will record the intervention assignment on the applicable case report form, if required. Potential bias will be reduced by central randomization.

6.4 Study Treatment Compliance

When subjects are dosed at the site, they will receive study treatment directly from the investigator or designee, under medical supervision. The date and time of each dose administered in the clinic will be recorded in the source documents and recorded in the eCRF. The dose of study treatment and study subject identification will be confirmed at the time of dosing by a member of the site staff other than the person administering the study treatment.

When subjects or caregivers administer study treatment(s) at home, the date, time, and quantity of each dose of study drug administered to each subject must be recorded by the subject or caregiver in a workbook provided for the study. The site staff will collect the workbook at scheduled clinic visits and transcribe this information to the appropriate eCRF.

These data will be used to assess compliance. Deviation(s) from the prescribed dosage regimen should be recorded in the eCRF. Treatment start and stop dates, including dates for treatment delays and/or dose reductions, should also be recorded in the eCRF.

6.5 Dose Modification

Subjects will receive pegvaliase at a concentration of 5.0 or 20.0 mg/mL. Pegvaliase will be administered subcutaneously at dose levels in the range of 2.5 to 60 mg. The minimum dose of pegvaliase is 2.5 mg/week. The maximum allowable daily dose of pegvaliase is 60 mg/day (for a maximum weekly total dose of 420 mg).

The duration of the Primary Treatment Phase (Part 1) is 72 weeks, during which pegvaliase treatment will be initiated for subjects randomized to the active (pegvaliase) treatment arm using an I/T/M dosing regimen. The recommended dosing schedule for subjects dosing with pegvaliase is shown in Table 6.5.1. Induction will consist of a 4-week period when subjects randomized to the active treatment arm will receive pegvaliase subcutaneously with a PFS at a fixed dose of 2.5 mg/week and during which the dosing regimen will not be modified in response to blood Phe concentration. Once subjects complete induction they will titrate up to a dose of 10 mg/day. The dosing frequency will be increased gradually during titration dosing to daily (7 days/week). During Maintenance Dosing the dose will be increased to 20 mg/day, 40 mg/day, and 60 mg/day depending upon individual blood Phe lowering efficacy (per the investigator's discretion). The dose should be increased to 40 mg/day if blood Phe is > 360 µmol/L after 24 weeks dosing at 20 mg/day and increased to 60 mg/day if blood Phe is > 360 µmol/L after 16 weeks dosing at 40 mg/day. If a subject's blood Phe is > 600 µmol/L after 16 weeks of continuous treatment with pegvaliase at 60 mg/day, pegvaliase should be permanently discontinued. BioMarin recommends that the target blood Phe levels should be based on individual needs, with a target blood Phe ≤ 600 µmol/L.

Duration between dose titration steps may be increased in response to AEs. Dose level reductions due to AEs or hypophenylalaninemia (confirmed with 2 consecutive blood Phe values < 30 µmol/L) may be made at any time during the study at the discretion of the investigator. Phe levels will be regularly monitored and adjustments in the pegvaliase dose and/or dietary intake of Phe will be required to mitigate the potential risk of chronic hypophenylalaninemia levels (< 30 µmol/L) in adolescent subjects.

Following completion of Part 1, subjects randomized to the active (pegvaliase) treatment arm will enter the Extension Phase (Part 2), during which subjects will continue dosing with pegvaliase at their optimal maintenance dose level for up to an additional 80 weeks in order to evaluate the long-term safety and efficacy of pegvaliase in adolescent subjects with PKU.

Following completion of Part 1, subjects randomized to the control (diet-only) treatment arm in Part 1 will repeat the Part 1 assessment schedule while initiating pegvaliase dosing from Weeks 73 through 145, after which they will receive pegvaliase for up to 8 additional weeks (through Week 153).

Table 6.5.1: Study Drug Dose Titration Schedule

Part 1 Dosing	Duration [a]	Dose (mg)	Frequency of Administration (Doses Per Week)
Induction	4 weeks	2.5	1
Titration	5 weeks	2.5	2
		10	1
		10	2
		10	4
		10	7
Maintenance	20 mg daily: 24 weeks [b] 40 mg daily: 16 weeks [b,c] 60 mg daily: maximum dose [c]	20	7
		40	7
		60	7

[a] Minimum duration prior to dose escalation. Dose escalation may be delayed depending on subject tolerability.

[b] Prior to initial dose escalation, the minimum amount of time at 20 mg/day is 24 weeks and the minimum amount of time at 40 mg/day is 16 weeks. The dose should be increased to 40 mg/day if blood Phe is > 360 µmol/L after 24 weeks dosing at 20 mg/day, and increased to 60 mg/day if blood Phe is > 360 µmol/L after 16 weeks dosing at 40 mg/day.

[c] If multiple injections are needed for a single dose, the injections should be administered at the same time of day and injection sites should be at least 5 cm away from each other. Doses should not be divided over the course of the day. If a subject's blood Phe is > 600 µmol/L after 16 weeks of continuous treatment with pegvaliase at 60 mg/day, pegvaliase should be permanently discontinued.

Following enrollment, subjects (and an adult caregiver) will be trained on how to administer study drug at home. Pegvaliase dosing will be conducted in a hospital setting for at least the first 8 weeks including titration up to 10 mg once daily, and for all subsequent dose escalations. The subject (and the adult caregiver) must demonstrate competency with injecting study drug in order to be permitted to administer study drug at home. Dosing at home will not be allowed until the principal investigator has assessed the subject's and adult observer's competency in proper study drug administration and recognition and appropriate management of the potential risks.

Throughout the study, an adult observer must be present during study drug administration and for a minimum of 1 hour following administration. An adult observer is defined as someone who will be present with the subject during and after pegvaliase administration, is able to recognize the signs and symptoms of an anaphylaxis episode, and is able to call for emergency medical support and administer epinephrine, if warranted. Administration of study drug may only be performed if this adult observer is present. Subjects and the adult observers who are observing them during study drug administration will be provided with information and extensive training regarding how to recognize a possible allergic reaction,

the severity of the reaction, and instructions on what to do if a reaction occurs. Home administration may not performed until the investigator has assessed the adult observer's competency in proper study drug administration and the recognition and appropriate management of potential risks.

Subjects will be provided with 2 epinephrine injectors and are required to carry both injectors with them at all times. Subjects and adult observers will be trained to recognize the signs and symptoms of anaphylaxis and, if these reactions occur, to call for emergency medical support and administer an epinephrine injector. For the duration of the study, each subject will be contacted weekly by study site personnel to monitor for administration problems and AEs (see Section 8.3.8.1 for details on the management of suspected anaphylaxis in this study).

All subjects receiving pegvaliase must be premedicated with an H1 antagonist, and an H2 antagonist, and an antipyretic approximately 2 to 3 hours prior to each dose until they reach the Maintenance Phase, defined as when subjects achieve blood Phe ≤600 µmol/L while they are on a stable dose of pegvaliase. Premedication may be considered during the Maintenance Phase at the investigator's discretion, eg, approximately 2 to 3 hours prior to study drug for 1 week upon reintroduction of study drug, upon resolution of an AE, following any dose interruption of ≥ 4 days, and for any dose increases in the Extension Phase. Subjects may also be premedicated at any other time in the study per investigator discretion.

This premedication regimen was successfully implemented in the Phase 3 adult clinical program. Subjects will be provided with a workbook to document the date and time of study drug injections, the injection site, premedication use, and suspected AEs.

If ≥ 4 consecutive doses are missed for reasons other than safety related issues, the investigator should obtain approval from the medical monitor prior to the subject resuming study drug.

6.5.1 Dosing Modifications in Response to Hypersensitivity Adverse Events

Dosing modifications in response to a hypersensitivity adverse event (HAE) depends on the NCI-CTCAE grade and suspected relationship study drug. Dosing instructions are provided in Table 6.5.1.1 (regardless of previous occurrence). Dosing modifications are per reported HAE, independent of prior reported HAEs.

Table 6.5.1.1: Dosing Instructions in Response to a Hypersensitivity Adverse Event

NCI-CTCAE Grade [a]	Related to Study Drug	Action with Study Drug			Individual Stopping Criteria Evaluation [d]	HRV Assessment [e]
		Maintain [b]	Reduce [c]	Interrupt [c]		
1	Yes or No	X	(X) Optional	(X) Optional		Investigator discretion
2	Yes or No	X	(X) Optional	(X) Optional		Investigator discretion
3	No				X Immediately consult with sponsor medical monitor	Investigator discretion
3 [d]	Yes			X	X Immediately consult with sponsor medical monitor	Yes (if within 24 hours of onset)
4 [d]	Yes or No			X	X Immediately consult with sponsor medical monitor	Yes (if within 24 hours of onset)

AE, adverse event; CTCAE, Common Terminology Criteria for Adverse Events, Version 5.0; HRV, Hypersensitivity Reaction Visit; NCI, National Cancer Institute.

[a] NCI-CTCAE grade determination is performed by the investigator and may be done either via telephone or clinic visit.

[b] The investigator will instruct the subject to maintain the study drug dose at the time of AE onset until improvement to Grade 1 or resolution (per investigator assessment in the clinic or via telephone).

[c] The study drug dose may be reduced or interrupted if necessary per investigator determination.

[d] If a subject has an NCI-CTCAE Grade ≥ 3 hypersensitivity AE that is related to study drug and is suspected to meet Brown's criteria for severe (Grade 3) in the judgment of the investigator and/or the sponsor's medical monitor, the subject will be permanently discontinued from study drug.

[e] If the investigator determines that the NCI-CTCAE Grade ≥ 3 hypersensitivity reaction is related to administration with study drug, the subject will be asked to return to the clinic within 24 hours of event onset for evaluation, including laboratory tests (chemistry, hematology, urinalysis, anti-pegvaliase IgE [sampling should be performed 8 to 24 hours after event onset and before the next dose of study drug], urine albumin/creatinine ratio, CRP, C3, and C4).

Once an HAE (other than an anaphylaxis episode) improves to Grade 1 or resolves, the study drug dose may be increased, maintained, or reduced, at the discretion of the investigator. If reduced, reductions should be from 60 to 40 mg/day, from 40 to 20 mg/day, or from 20 to 10 mg/day, whichever is appropriate.

6.6 Treatment after the End of the Study

There is no treatment following completion of the study.

6.7 Overdose

For this study, any dose of pegvaliase greater than the prescribed dose will be considered an overdose.

BioMarin does not recommend specific treatment for an overdose. In the event of an overdose, the investigator should contact the BioMarin medical monitor immediately.

6.8 Concomitant Therapy

Any medication or vaccine (including over-the-counter or prescription medicines, vitamins, and/or herbal supplements) that the subject is receiving at the time of enrollment or receives during the study must be recorded on the designated eCRF along with:

- Reason for use
- Dates of administration including start and end dates
- Dosage information including dose and frequency

The medical monitor should be contacted if there are any questions regarding concomitant or prior therapy. The investigator may prescribe additional medications, deemed necessary to provide adequate prophylactic or supportive care, during the study, as long as the prescribed medication is not prohibited by the protocol (refer to Section 5.2). In the event of an emergency, any needed medications may be prescribed without prior approval, but the medical monitor must be notified of the use of any contraindicated medications immediately thereafter. Any concomitant medications added or discontinued during the study should be recorded on the eCRF. Medications should, whenever possible, not be recorded in the electronic database with a frequency of PRN.

6.8.1 Excluded Concomitant Medications

The following medications are prohibited starting at the time specified and until the end of the study:

- Any medication that is intended to treat PKU, including the use of large neutral amino acids, within 14 days prior to the administration of study drug on Day 1.
- Any injectable drugs containing polyethylene glycol (PEG; other than pegvaliase), including medroxyprogesterone injection, within 3 months prior to the start of Screening/Run-in and during study participation with the exception of COVID-19 vaccinations. Please contact the medical monitor prior to administering COVID-19 vaccinations.
- Use of any investigational product or investigational medical device within 30 days prior to Screening/Run-in or requirement for any investigational agent prior to completion of all scheduled study assessments.

In addition, if a subject is on medication for ADHD, depression, or other psychiatric disorder, the dose must be stable for ≥ 8 weeks prior to enrollment and must continue to be stable throughout the study unless a change is medically indicated.

6.8.2 Permitted and/or Required Concomitant Medications

All prescription and over-the-counter medication including herbal supplements and medical food taken by a subject for 30 days before screening are recorded on the designated CRF. Psychiatric medications and their dosage should remain consistent throughout the study unless a change is medically indicated. No new psychiatric medication should be initiated during the study unless medically indicated. No psychotherapy should be initiated during the study unless medically indicated. The investigator may prescribe additional medications during the study as long as the prescribed medication is not prohibited by the protocol (see Section 6.8.1). In the event of an emergency, any needed medications may be prescribed without prior approval but the medical monitor must be notified of the use of any contraindicated medication immediately thereafter. Any concomitant medication added or discontinued during the study should be recorded on the appropriate CRF.

All subjects receiving pegvaliase will be required to premedicate with an H1 antagonist, and an H2 antagonist, and an antipyretic approximately 2 to 3 hours prior to each dose of study drug until they reach the Maintenance Phase, defined as when subjects achieve blood Phe ≤600 µmol/L while they are on a stable dose of pegvaliase. Premedication may be considered during the Maintenance Phase at the investigator's discretion. For further information regarding premedication, refer to Section 6.5.

7 DISCONTINUATION OF STUDY TREATMENT AND SUBJECT WITHDRAWAL FROM STUDY

7.1 Discontinuation of Study Treatment

In rare instances it may be necessary for a patient to permanently discontinue study treatment. If a subject's blood Phe is > 600 µmol/L after 16 weeks of continuous treatment with pegvaliase at 60 mg/day, pegvaliase should be permanently discontinued.

If study treatment is permanently discontinued, the subject will be asked to continue to provide consent at the time of treatment discontinuation to remain in the study and undergo the scheduled study assessments. Refer to the SoA for data to be collected at the time of discontinuation of study treatment and follow-up, and for any further evaluations that need to be completed.

If the subject declines to provide consent to remain in the study, the study completion visit assessments should be performed as detailed in the SoA (Section 1.3).

7.2 Subject Withdrawal from the Study

A subject may withdraw from the study at any time at his or her own request, or may be withdrawn at any time at the discretion of the investigator for safety, behavioral, compliance, or administrative reasons.

At the time of discontinuing from the study, if possible, an early termination (ET) visit should be conducted, as shown in the SoA (Section 1.3).

The subject will be permanently discontinued both from the study treatment and from the study at that time.

If the subject withdraws consent for disclosure of future information, the sponsor may retain and continue to use any data collected before such a withdrawal of consent.

If a subject withdraws from the study, BioMarin will only use the remaining samples to complete study procedures as described in Section 8 and no new samples will be collected. If a subject no longer wants the remaining samples to be used for future research, he/she shall inform the study investigator or institution accordingly and BioMarin will only use the remaining samples for health authority requests related to the activities in Section 8, not for future research. The investigator must document this in the site study records.

7.3 Lost to Follow-Up

A subject will be considered lost to follow-up if he or she repeatedly fails to return for scheduled visits and cannot be contacted by the site.

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

- The site must attempt to contact the subject to reschedule the missed visit as soon as possible, counsel the subject on the importance of maintaining the assigned visit schedule, and ascertain whether or not the subject wishes to and/or should continue in the study.
- Before a subject is deemed lost to follow up, the investigator or designee must make every effort to regain contact with the subject (where possible, 3 telephone calls and, if necessary, a certified letter to the subject's last known mailing address or local equivalent methods). These contact attempts should be documented in the subject's medical record.
- Should the subject continue to be unreachable, he or she will be considered to have withdrawn from the study.

Discontinuation of specific sites or of the study as a whole are handled as part of Appendix 1, Section 10.1.9.

8 STUDY ASSESSMENTS AND PROCEDURES

An ICF must be signed and dated by the subject's legally authorized representative, the investigator or designee, and a witness (if required) before any study-related procedures are performed.

All screening evaluations must be completed and reviewed to confirm that potential subjects meet all eligibility criteria. The investigator will maintain a screening log to record details of all subjects screened and to confirm eligibility or record reasons for screening failure, as applicable. Procedures conducted as part of the subject's routine clinical management (eg, blood count) and obtained before signing of the ICF may be utilized for screening or baseline purposes provided the procedures met the protocol-specified criteria and were performed within the time frame defined in the SoA.

Potential subjects will enter the Screening/Run-in period, which will last 4 weeks and include 2 assessments of blood Phe concentration 7 to 10 days apart. Diet diaries should be completed for the 3 days preceding each of the blood Phe assessments. Following confirmation that the 2 blood Phe results are > 600 µmol/L and all other eligibility criteria have been met, subjects may be enrolled in the study on Day 1. Protocol exemptions for eligibility are not allowed. Following enrollment, subjects will be randomized 2:1 to the active (pegvaliase) or control (diet-only) treatment arm of the study.

In the Primary Treatment Phase (Part 1), subjects in the active (pegvaliase) and control (diet-only) treatment arms will follow the same visit schedules and perform the same assessments except that the control subjects will not be receiving pegvaliase so will not have PK draws or immunogenicity assessments. The timing of Part 1 assessments for active and control subjects is provided in Table 1.3.1 and Table 1.3.3, respectively.

The primary efficacy endpoint, the change from baseline blood Phe, is assessed following 72 weeks on study (the end of Part 1). Beginning Week 73, subjects in the active treatment arm will continue to receive pegvaliase in the Extension Phase (Part 2) for up to an additional 80 weeks (through Week 153). The timing of Part 2 assessments for active treatment subjects is provided in Table 1.3.2.

Subjects in the control (diet-only) treatment arm will initiate pegvaliase treatment beginning Week 73 and, from Weeks 73 through 145, will follow the same dosing and assessment schedule that the active subjects followed from Weeks 1 through 73, after which they will receive pegvaliase for 8 additional weeks (through Week 153). The timing of Part 2 assessments for control subjects is provided in Table 1.3.4. All the assessments, dietary rules, and other restrictions that applied to active subjects in the Primary Treatment Phase (Part 1)

apply to control subjects in Part 2 until the Week 145 visit when change from baseline blood Phe for these subjects will be assessed after 73 weeks of pegvaliase treatment.

The last scheduled visit, which includes study drug administration, is at Week 153 for subjects randomized to both the active (pegvaliase) and control (diet-only) treatment arms. Subjects should perform the Study Completion (SC) Visit 4 weeks after the last dose of study drug or after completion of study participation, whichever occurs last. For subjects who terminate from the study early, the Early Termination (ET) visit should occur within 4 weeks after the last study drug dose or after withdrawal from study participation, whichever occurs last. If study drug is discontinued before study completion, the investigator will ask the subject to remain in the study to continue study visits and assessments, provided the subject's health, safety, and welfare would not be detrimentally affected.

Immediate safety concerns should be discussed with the sponsor (medical monitor) immediately upon occurrence or awareness to determine if the subject should continue or discontinue study treatment.

Adherence to the study design requirements, including those specified in the SoA, is essential and required for study conduct.

8.1 Efficacy Assessments

Planned timepoints for all efficacy assessments are provided in the SoA (refer to Section 1.3).

8.1.1 Blood Phe Level

The primary efficacy endpoint is change in blood Phe concentration from treatment naïve baseline following 72 weeks on study (see Section 9.4.2). Blood samples for blood Phe analysis are to be drawn according to the SoA (see Section 1.3). Monitoring of blood Phe levels is the cornerstone of diagnosis and treatment of PKU patients. Guidelines for the treatment of PKU are based on blood Phe levels (Vockley 2014; van Wegberg 2017), and clinicians and patients use blood Phe concentration to assess and monitor PKU disease progression and control.

Subjects must fast for at least 2.5 hours prior to blood Phe assessments to lessen the effect of food intake on blood Phe concentration. Samples for blood Phe concentration will be analyzed by a central laboratory.

8.1.2 Total Dietary Protein Intake, Dietary Monitoring, and Dietary Protocol Restrictions

Protein from medical foods (Phe-free amino acid fortified food sources) and protein from intact foods (any other food sources containing Phe) are collectively referred to as total dietary protein. Although total dietary protein intake is not an efficacy endpoint in this study, it is an important outcome measure that must remain stable for the primary endpoint to be accurately assessed and under the supervision of a dietician or adequately trained designee per the investigator during study participation. Specifically, to accurately assess the primary endpoint comparison between the active (pegvaliase) and control (diet-only) treatment arms on the change from baseline in blood Phe concentration following 72 weeks on study, total protein intake must remain stable for subjects in both treatment arms during this period. It is essential that subjects maintain stable protein intake to ensure that the measured changes in their blood Phe are attributable to study treatment rather than changes in their dietary protein intake.

A subject's diet is monitored using a 3-day diet diary. The 3-day dietary record (diet diary) is completed by subjects, brought to clinic visits for review, and maintained with the study source documents. Subjects record all food, beverages, special low-protein foods, and medical foods consumed for 3 consecutive days prior to the scheduled clinic visit and data are analyzed by the nutritional software (Metabolic Pro®) for total kcals, protein, Phe, tyrosine, and the percentage of daily recommended intake provided for protein, Phe, tyrosine, vitamins, and minerals.

A dietitian under investigator supervision is required to monitor and manage the subject's diet for the entire duration of the study, though an investigator or designee may also perform this role. Subjects will be provided with 3-day diaries in which all dietary protein intake (including medical food and/or intact food) must be recorded for 3 consecutive days immediately prior to each scheduled clinic visit for review with the dietitian. The subject may be asked to repeat the 3-day diary collection if the dietitian or investigator/designee considers the diary has not been completed accurately; in such circumstances, study visits will be rescheduled. All subjects will be provided the option of tyrosine supplementation (500 mg, 3 times per day with meals) at the discretion of the investigator.

The change from baseline in total protein intake at Week 73 will be compared as a secondary endpoint. For this endpoint, baseline protein intake is defined as the average of protein intake values derived from diet diaries collected during Screening/Run-in. During Screening/Run-in, 3-day diet diaries are completed 3 times, each time covering the 3 days periods prior to blood Phe assessments (and collected on the day of those assessments). The

3 Screening/Run-in blood Phe assessments are scheduled to occur (1) at Day -25 to Day -23; (2) 7 to 10 days later (ie, in the Day -18 to Day -13 window); and (3) at Day 1 pre-dose. Thus, the 3 day diet assessments will assess diet for (1) the 3 days within the Day -28 to Day -23 window immediately prior to the first blood Phe assessment; (2) the 3 days within the Day -21 to Day -15 window immediately prior to the second blood Phe assessment; and (3) for Days -3 to Day 1.

Subjects will be instructed not to change their total dietary protein intake (medical food and/or intact food) during the Screening/Run-in and Primary Treatment Phase (Part 1) of the study. However, modifications to a subject's diet and reductions in study drug dose level must be implemented if blood Phe levels are reduced to < 30 µmol/L and confirmed upon recheck (performed within approximately 2 weeks) (see Section 8.1.2.1).

In Part 2, dietary protein intake may be modified for subjects randomized to active (pegvaliase) treatment if blood Phe concentration is ≤ 360 µmol/L for a minimum of 4 weeks. Should that occur, subjects may adjust dietary protein intake based on individual subject response to pegvaliase and guidance from the investigator or designee (see Section 8.1.2.2). Subjects randomized to the control (diet-only) treatment arm must continue to maintain dietary protein intake consistent with baseline through Week 145 except in the case of blood Phe levels < 30 µmol/L confirmed upon recheck (see Section 8.1.2.1).

Baseline total dietary protein intake will be used as the comparator for subsequent 3-day diet diary entries. Subjects will be required to maintain dietary protein intake levels that are consistent with their baseline levels for the entire duration of the study, except as otherwise specified above, with a consistent diet defined as one in which the intact protein changes are < 15% from baseline and the medical food protein changes are < 15% from baseline.

If a subject demonstrates ≥ 15% change from baseline intake of total intact protein and/or medical food protein, the dietitian (or investigator/designee) will counsel the subject to resume their baseline diet and may perform another 3-day diet record and blood Phe concentration assessment in 2 weeks. If the subject demonstrates ≥ 15% increase in intake of protein from intact foods and/or medical food at the next scheduled study visit, a discussion with the medical monitor may be required to discuss further actions related to non-adherence with consistent dietary protein intake throughout the study, at the discretion of the investigator.

If dietary protein intake is determined to be inconsistent with baseline values, the study site dietitian (or investigator/designee) should counsel the subject on how to return to baseline values by modifying intake of protein from intact foods and/or medical food. If diet changes have been implemented, a blood Phe concentration assessment and 3-day diet record should

be collected 2 weeks after implementation, in addition to all regularly scheduled assessments. Any changes must also be documented on the appropriate eCRF.

8.1.2.1 Diet Modification for Blood Phe Levels Less than 30 µmol/L

In addition to protocol required dose reductions (Section 6.5), modifications to subject's diet should be performed if blood Phe levels are < 30 µmol/L and confirmed upon repeat blood Phe measurement (performed within approximately 2 weeks). Subjects will receive counseling for modification of intake of intact protein and medical foods based on intake at the time of blood Phe assessment, as follows:

- If it is determined that a subject is consuming < 2x their recommended level of intact protein per Dietary Reference Intake (DRI) guidelines (1.6 g/kg/day), the dietitian may instruct the subject to increase their intact protein by 20 grams/day and decrease their medical food protein by 20 grams/day.
- If it is determined that a subject is consuming ≥ 2x their recommended level of intact protein per DRI guidelines, the dietitian may instruct the subject to increase their intact protein by 10 grams/day and decrease their medical food protein by 10 grams/day.

Further increases in protein intake as per above are allowed if blood Phe levels are < 30 µmol/L in order to achieve 2 x DRI guidelines (1.6 g/kg/day) of intact protein intake. This increased intake will now be the new reference baseline for dietary protein intake for future dietary assessments. Medical food may be discontinued once the dietitian determines that the essential amino acids meet the appropriate levels for age.

8.1.2.2 Dietary Changes for Blood Phe ≤360 µmol/L in Extension Phase (Subjects Randomized to Pegvaliase)

Once subjects initially randomized to active (pegvaliase) treatment enter the Extension Phase (Part 2) of the study, dietary protein intake may be modified if blood Phe concentration reaches ≤ 360 µmol/L for a minimum of 4 weeks. If diet is modified, it is recommended to increase protein from intact foods in 10 or 20 gram increments depending on intact protein intake, and decrease protein from medical food by a similar amount, 10 or 20 grams unless the subject is already consuming the appropriate protein levels for age (World Health Organization 2007). Medical food may be discontinued once the dietitian or investigator/designee determines that the essential amino acids provided from intact protein foods meet the appropriate levels for age.

From the beginning of the Extension Phase (Part 2), subjects initially randomized to active (pegvaliase) treatment who have achieved blood Phe > 30 to ≤360 µmol/L may have their medical food intake reduced or discontinued and intact protein intake increased, in

accordance with Table 8.1.2.2.1. Implementation of dietary adjustments will be performed in consultation with the site dietitian.

Table 8.1.2.2.1: Dietary Adjustment Algorithm in Part 2 for Subjects Randomized to Active (Pegvaliase) Treatment

Phe Category [a]	Protein Intake from Intact Food Sources [b]	Action	
		Protein Intake from Intact Food Sources	Protein Intake from Medical Food
> 30 [d] to ≤ 360 µmol/L	> 2 × RDI (> 1.7 g/kg)	Maintain or reduce by 10 g/day [c]	Discontinue [c]
	> 1 × to 2 × RDI (> 0.8 to 1.7 g/kg)	Maintain	Discontinue [c]
	0.5 × to 1 × RDI (0.4 to 0.8 g/kg)	Increase 10 g/day [c]	Decrease 10 g/day [c]
	< 0.5 × RDI (< 0.4 g/kg)	Increase 20 g/day [c]	Decrease 20 g/day [c]

RDI, recommended daily intake.

[a] Based on results of the previous blood Phe assessment; blood Phe reductions may not be observed until the subject is titrated to an effective dose.

[b] RDI (0.83 g/kg) is based on the joint FAO/WHO/UNU Expert Consultation on Protein and Amino Acid Requirements in Human Nutrition (World Health Organization 2007).

[c] The resulting adjusted dietary protein intake will become the new reference baseline for dietary protein intake for future dietary assessments.

[d] If a subject experiences blood Phe levels < 30 µmol/L on two consecutive blood tests, refer to the action to take in the event of blood Phe ≤ 30 µmol/L (Section 8.1.2.1).

8.1.3 Exploratory Assessments

8.1.3.1 Neurocognitive Assessments

The ADHD-RS IV and BRIEF instruments will be used to evaluate the effect of pegvaliase treatment on neurocognitive outcomes in adolescent subjects with PKU. The same parent/guardian must complete both the ADHD-RS IV and the BRIEF at all scheduled time points.

8.1.3.1.1 The ADHD Rating Scale IV Inattention Subscale (ADHD-RS)

The ADHD-RS IV (American Psychiatric Association 2013; DuPaul 1998) will be used to assess inattention and hyperactivity symptoms among the adolescents enrolled in this study. The ADHD-RS is derived directly from diagnostic criteria delineated in the DSM, is well validated, and has been extensively used in a broad array of disease classes, including PKU (Gassio 2010; Wyrwich 2016). The ADHD-RS IV was the secondary endpoint in the

BioMarin Phase 3 pegvaliase study (165-302) in which improvement in inattention symptoms was associated with reduction in mean blood Phe levels (Thomas 2018).

The ADHD-RS IV instrument is completed independently by the parent/guardian. The same parent/guardian must complete the scale at each scheduled time point. The instrument consists of 2 nine-item scales that evaluate symptom severity for inattention and hyperactivity/impulsivity, respectively, during the previous month. Each scale is scored separately. Each item is rated on a 4-point severity scale (0, none; 1, mild; 2, moderate; 3, severe); scores of 2 or 3 on an individual item indicate a symptom. The PKU literature suggests that inattentive symptoms are more prevalent than hyperactivity and impulsivity symptoms among individuals with PKU; thus, the inattentive sub-scale is considered the most relevant and will be included in analyses (Bilder 2016).

A vendor specializing in cognitive testing provides study staff training for the investigator-rated ADHD-RS IV administration.

8.1.3.1.2 Behavior Rating Inventory of Executive Function (BRIEF)

The BRIEF is an 86-item measure designed to assess executive function skills in children 5 to 18 years of age (Gioia 2002). The BRIEF was developed to address the limitations of available assessments in examining real-word expressions of behaviors related to executive function. The BRIEF is intended to provide a standard method of asking multiple raters about executive function in daily life in a manner that is not specific to any particular disease. Because it is non-disorder-specific, BRIEF assesses executive function behaviors in children and adolescents experiencing a wide range of difficulties, such as those related to learning, attention, brain injuries, development disorders, and various psychiatric conditions and medical issues. The BRIEF is most often used to assess attention deficit hyperactivity disorder.

The BRIEF instrument (completed by the parent/guardian) contains 86 items in 8 non-overlapping clinical scales and 2 validity scales. The same parent/guardian must complete the BRIEF at each scheduled time point. These scales form 2 indexes: (1) Behavior Regulation (3 scales) and (2) Metacognition (5 scales), as well as a Global Executive Composite score which takes into account all of the clinical scales and represents the adolescent's overall executive function.

8.2 Safety Assessments

Planned time points for all safety assessments are provided in the SoA (Section 1.3).

8.2.1 Physical Examination

Physical examinations (full or brief) should be performed at the timepoints indicated in the SoA (Section 1.3):

- A full physical examination will include, at a minimum, general appearance (head, eyes, ears, nose, and throat) and an assessment of cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, musculoskeletal, and neurologic systems.
- A brief physical examination may be performed at the discretion of the investigator based on the subject's clinical condition and may include, but is not limited to, general appearance and an assessment of cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, musculoskeletal, and neurologic systems.

Investigators should pay special attention to clinical signs related to previous serious illnesses.

Height and weight will also be measured and recorded at the timepoints indicated in the SoA (Section 1.3).

8.2.2 Vital Signs

Vital signs include respiration rate, temperature, and single measurements of heart rate and seated diastolic and diastolic blood pressure. Any clinically significant change in vital signs will be recorded as an AE. Blood pressure and pulse measurements should be preceded by at least 5 minutes of rest for the subject in a quiet setting without distractions (eg, television, cell phones). Blood pressure and pulse measurements will be assessed with a completely automated device. Manual techniques will be used only if an automated device is not available.

Results will be recorded on the eCRF. Any abnormal vital sign assessments should be repeated once, and repeat value should be recorded in the eCRF.

8.2.3 Electrocardiograms

Electrocardiograms (ECGs) will be performed at Screening, Baseline, and SC/ET, as shown in the SoA (Section 1.3). Single 12-lead ECG will be obtained using an ECG machine that automatically calculates the heart rate and measures PR, QRS, QT, and QTc intervals.

8.2.4 Clinical Safety Laboratory Assessments

See Appendix 2, Section 10.2 for the table of clinical laboratory tests to be performed and refer to the SoA (Section 1.3) for the timing and frequency.

The investigator must review the laboratory report and document this review. The laboratory reports must be filed with the source documents. If known, the underlying diagnosis associated

with abnormal clinical laboratory test results that are considered clinically significant by the investigator should be recorded on the AE eCRF. Clinically significant abnormal laboratory findings are those which are not associated with the underlying disease, unless judged by the investigator to be more severe than expected for the subject's condition.

All abnormal laboratory tests with values considered clinically significant per the investigator during participation in the study and 30 days after the last dose of study treatment should be repeated until the values return to normal or baseline or are no longer considered clinically significant by the investigator.

In the event of elevated urinary protein on a test result, a repeat urinalysis should be performed. This repeat urine sample must be performed in the morning at the first or second morning void to allow for accurate test results and may be performed by a home healthcare nurse. Subjects with a confirmed urine/albumin creatinine ratio of ≥ 100 mg/g should be referred to a nephrologist for consultation if results were within normal range at Baseline. Subjects who had elevated results at Baseline followed by a confirmed subsequent increase of ≥ 100 mg/g from Baseline should also be referred to a nephrologist for consultation.

If clinically significant values do not return to normal/baseline within a period of time judged reasonable by the investigator, the etiology should be identified and the medical monitor notified.

All protocol-required laboratory assessments, as defined in Appendix 2, Section 10.2, must be conducted in accordance with the laboratory manual and the SoA.

If laboratory values from non-protocol specified laboratory assessments performed at the institution's local laboratory require a change in subject management or are considered clinically significant by the investigator (eg, SAE or AE or dose modification), then the results must be recorded in the eCRF.

8.2.5 Pregnancy Testing

Based on findings in studies of pregnant animals without PKU treated with pegvaliase-pqpz, pegvaliase may cause fetal harm when administered to a pregnant woman. Because the risks of taking study drug during pregnancy and breastfeeding in humans are unknown, subjects must not take study drug if they are trying to conceive, are pregnant, or are breastfeeding. From Day –28 until the end of the study, sexually active subjects must use 2 acceptable methods of contraception as outlined in the inclusion criteria section of this protocol (Section 5.1). Subjects who are confirmed to be pregnant by a serum pregnancy test and are temporarily off study drug are not required to perform the scheduled urine pregnancy tests.

In addition to contraception requirements, females of child-bearing potential are required to have urine pregnancy tests performed at the timepoints specified in the SoA (Section 5.1). Urine pregnancy test should be performed by the local laboratory. A serum pregnancy test will be performed by the central laboratory in the event of a positive or equivocal urine pregnancy test result.

Refer to Section 8.3.5 for details on the reporting procedures in the event of pregnancy.

8.3 Adverse Events and Serious Adverse Events

The definitions of an AE or SAE can be found in Appendix 3, Section 10.3.

AEs will be reported by the subject (or, when appropriate, by a caregiver, surrogate, or the subject's legally authorized representative).

The investigator and any medically qualified designees are responsible for identifying, documenting, and recording events that meet the definition of an AE or SAE and remain responsible for following up AEs that are serious, considered related to the study treatment or study procedures, or that caused the subject to discontinue the study treatment (refer to Section 7).

8.3.1 Time Period and Frequency for Collecting AE and SAE Information

Control (diet-only) treatment arm subject reporting:

- After informed consent to Day 1, only SAEs related to protocol mandated procedures will be collected.
- All AEs, SAEs, AESI, pregnancy and device related events will be collected from Day 1 through 4 weeks after last dose of study treatment or the study completion visit, whichever is longer.
- Non serious events that occur after informed consent but prior to Day 1 will be recorded on the Medical History/Concurrent Medical Conditions eCRF, not the AE eCRF.

Active (pegvaliase) treatment arm subject reporting:

- After informed consent through the first dose of study treatment, only SAEs related to protocol mandated procedures will be collected.
- All AEs, SAEs, AESI, pregnancy, and device-related events will be collected after the first dosed of study treatment through 4 weeks after the last dose of study treatment or the study completion visit, whichever is longer.
- Non serious events that begin after informed consent but before study treatment will be recorded on the Medical History/Concurrent Medical Conditions eCRF, not the AE eCRF.

All SAEs and AESIs must be recorded and reported to the sponsor or designee immediately; under no circumstances should this exceed 24 hours after the site becomes aware of the event, as indicated in Appendix 3, Section 10.3. The investigator must submit any updated SAE data to the sponsor within 24 hours of it being available.

Investigators are not obligated to actively seek AE or SAE information after conclusion of study participation. However, if the investigator learns of any SAE, including a death, at any time after a subject has been discharged from the study and he/she considers the event to be reasonably likely to be related to the study treatment or study participation, the investigator must promptly notify the sponsor.

8.3.2 Method of Identifying AEs and SAEs

The method of recording, evaluating, and assessing causality of AEs and SAEs and the procedures for completing and transmitting SAE reports are provided in Appendix 3, Section 10.3.

Care will be taken not to introduce bias when identifying AEs and/or SAEs. Open-ended and non-leading verbal questioning of the subject is the preferred method to inquire about AE occurrences.

8.3.3 Follow-up of AEs and SAEs

After the initial AE/SAE report, the investigator is required to proactively follow each subject at subsequent visits/contacts. All SAEs and AESIs (as defined in Section 8.3.8) will be followed until resolution, stabilization, the event is otherwise explained (ie, not related to study drug and/or study participation), or the subject is lost to follow-up (as defined in Section 7.3). Further information on follow-up procedures is provided in Appendix 3, Section 10.3.

8.3.4 Regulatory Reporting Requirements for SAEs

Prompt notification by the investigator to the sponsor of a SAE is essential so that legal obligations and ethical responsibilities towards the safety of subjects and the safety of a study treatment under clinical investigation can be met.

The sponsor has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a study treatment under clinical investigation. The sponsor will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority, Institutional Review Boards (IRB)/Independent Ethics Committees (IEC), and investigators.

Expedited safety reports must be prepared for suspected unexpected serious adverse reactions according to global regulatory requirements and sponsor policy and forwarded to investigators.

An investigator who receives an expedited safety report or other specific safety information from the sponsor will review and then file it along with the IB and will notify the IRB/IEC as appropriate.

8.3.5 Pregnancy

The importance of pregnancy prevention for study subjects and their partners will be communicated to all study subjects with regular reminders during the study.

Any female subject who becomes pregnant while participating in the study will temporarily discontinue study treatment.

Details of all pregnancies in female subjects and, if indicated, female partners of male subjects, will be collected after the start of study treatment and until 4 weeks after the last dose of study treatment.

While pregnancy itself is not considered to be an AE or an SAE, any pregnancy complication or elective termination of a pregnancy for medical reasons will be reported as an AE or SAE.

Abnormal pregnancy outcomes (eg, spontaneous abortion, fetal death, stillbirth, congenital anomalies, ectopic pregnancy), as well as any hospitalizations associated with pregnancy complications, unplanned caesarean section, and delivery complications should always be classified as an SAE (as the sponsor considers these to be medically significant), recorded on the eCRF, and expeditiously reported to the sponsor as an SAE following the procedures outlined in Appendix 3, Section 10.3.4.

The subject (or pregnant female partner) will be followed to determine the outcome of the pregnancy. The investigator will collect follow-up information on the subject (or pregnant female partner) and the neonate, and the information will be forwarded to the sponsor.

Any post-study pregnancy-related SAE considered reasonably related to the study treatment by the investigator should be reported to the sponsor as described in Section 8.3.4. While the investigator is not obligated to actively seek this information in former study subjects (or their pregnant female partners), he or she may learn of an SAE through spontaneous reporting.

Pregnancy reporting requirements to BioMarin are shown in Table 8.3.5.1.

Table 8.3.5.1: Pregnancy Reporting to BioMarin

Pregnancy Reporting to BioMarin
• Pregnancy and Pregnancy Outcome information will be entered into the electronic data collection system. • Pregnancy and Pregnancy Outcome information will be reported to BPV via the study specific Pregnancy Report and Pregnancy Outcome paper report forms. The site will complete the forms and submit via facsimile or email without 24 hours of awareness. • Abortion, Stillbirth, Fetal Congenital Anomaly and any other event meeting seriousness criteria must be reported as an SAE via the study electronic data collection system.

Male Subjects with Partners Who Become Pregnant

The investigator will attempt to collect pregnancy information on any male subject's female partner who becomes pregnant while the male subject is in this study. This applies only to male subjects who receive pegvaliase.

After obtaining the necessary signed informed consent from the pregnant female partner directly, the investigator will record pregnancy information on the appropriate form and submit it to the sponsor within 24 hours of learning of the partner's pregnancy. The female partner will also be followed to determine the outcome of the pregnancy. Information on the status of the mother and child will be forwarded to the sponsor. Generally, the follow-up will be no longer than 6 to 8 weeks following the estimated delivery date. Any termination of the pregnancy will be reported regardless of fetal status (presence or absence of anomalies) or indication for the procedure.

8.3.6 Cardiovascular and Death Events

All deaths that occur during the AE reporting period, regardless of attribution, will be recorded on the AE eCRF and expeditiously reported to the Sponsor as an SAE.

When recording a death, the event or condition that caused or contributed to the fatal outcome should be recorded as the single medical concept on the eCRF. If the cause of death is unknown and cannot be ascertained at the time of reporting, record "Unexplained Death" or "Death of Unknown Cause" on the eCRF.

8.3.7 Disease-Related Events and/or Disease-Related Outcomes Not Qualifying as AEs or SAEs

The following disease-related event (DRE) is common in subjects with PKU and can be serious:

- Hyperphenylalaninemia (Medical Dictionary for Regulatory Activities terminology [MedDRA] term: amino acid increased)

Because this event is typically associated with the disease under study, it will not be reported according to the standard process for expedited reporting of SAEs even though the event may meet the definition of a SAE. The elevated blood Phe levels should be recorded in the central laboratory database as per standard practice. These DREs will be monitored on a routine basis.

If either of the following conditions applies, then the event must be recorded and reported as an SAE (instead of a DRE):

- The event is, in the investigator's opinion, of greater intensity, frequency, or duration than expected for the individual subject; OR
- The investigator considers that there is a reasonable possibility that the event was related to study treatment.

8.3.8 Adverse Events of Special Interest

The following adverse events of special interest (AESI) must be reported to BPV within 24 hours of site awareness, irrespective of seriousness, severity, or causality to facilitate real-time monitoring and assessment. All AESI must be reported using the SAE form regardless of seriousness:

- Acute systemic hypersensitivity reaction, anaphylactic, or anaphylactoid reactions (refer to Appendix 5, Section 10.5)
- Angioedema
- Serum sickness
- Severe injection site reaction defined as an injection site reaction that is considered CTCAE Grade ≥ 3
- Hypophenylalaninemia events (defined as blood Phe < 30 µmol/L on 2 consecutive measurements)
- Arthralgia that is either persistent (AE duration > 6 months), severe (CTCAE Grade ≥ 3), or that requires treatment with a steroid (excluding topical)

8.3.8.1 Anaphylaxis (Acute Systemic Hypersensitivity Reaction)

All potential anaphylactic events, however reported, will be reviewed by BioMarin. When an event occurs which an investigator believes is possibly anaphylaxis, in addition to reporting the event to BPV within 24 hours of site awareness, it should be reported to the sponsor's medical monitor within 24 hours of site awareness.

In this protocol the term anaphylaxis is used to denote the important identified risk: acute systemic hypersensitivity reaction (see Section 2.3.1). An acute systemic hypersensitivity reaction (referred to as anaphylaxis in this protocol) is defined as an episode meeting National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network (NIAID/FAAN) criteria according to an internal BioMarin assessment performed by the Internal Data Safety Review Board. The Internal Safety Data Review Board, consisting of a representative from BioMarin clinical sciences, safety, and biostatistics departments, will review all cases of potential anaphylaxis to determine which of these events meet the criteria for an acute systemic hypersensitivity reaction as summarized in Appendix 5, Section 10.5.2.

In the clinical study report, analyses of anaphylaxis will include both the definition used in this protocol of an acute systemic hypersensitivity reaction and the FDA's definition of anaphylaxis, developed by the Division of Pulmonary, Allergy, and Rheumatology Products (DPARP) and used by BioMarin in the Palynziq US prescribing information (see the SAP for further details).

8.3.8.1.1 Individual Stopping Criteria Evaluation for Adverse Events During Pegvaliase Treatment

A subject who has an anaphylaxis episode that is suspected to meet Brown's criteria for severe (Grade 3) and that is, in the judgment of the investigator and/or the sponsor's medical monitor, related to study drug, will be permanently discontinued from study drug. Clinically severe hypersensitivity (Grade 3) per Brown's criteria is defined as significant hypoxia, hypotension or neurologic compromise that is life-threatening or required treatment to prevent a life-threatening event (Brown 2004):

- Cyanosis or $SpO_2 \leq 92\%$
- Hypotension with SBP < 90 mmHg
- Neurologic alteration: confusion, loss of consciousness, collapse, incontinence

In addition, a subject with a recurrent anaphylaxis episode of any severity that is, in the judgment of the investigator and/or the sponsor's medical monitor, related to study drug will be permanently discontinued from treatment.

8.3.8.1.2 Response to Anaphylaxis Episodes

If a subject experiences any symptoms suggestive of an anaphylaxis episode (as taught to the subject and adult observer in the initial and ongoing training on this risk), they will be instructed to inject epinephrine, to seek immediate medical attention, *and* to inform the investigator.

If the investigator suspects that an AE is anaphylaxis as defined by NIAID/FAAN, the subject will be assessed in the clinic and the sponsor's medical monitor must be immediately notified. Laboratory assessments for suspected anaphylaxis events described in the SoA (Section 1.3) under Hypersensitivity Reaction Visit (HRV) and in Table 6.5.1.1 should be performed prior to the next administration of study drug (if applicable) and should include anti-pegvaliase IgE (for optimal results, sampling should be performed 8 to 24 hours after event onset). If the investigator determines it is safe for the subject to resume dosing with study drug following resolution of anaphylaxis at any time during the study (excepting episodes requiring subject discontinuation, as outlined above), the following steps are required:

- At least the first dose administered after resolution of anaphylaxis is to be given at the clinic with equipment for emergency resuscitation (including epinephrine) within easy access.
- The subject must be premedicated with an H1 antagonist, and an H2 antagonist, and an antipyretic (eg, acetaminophen) approximately 2 to 3 hours prior to each dose of study drug for at least 1 week upon resumption of dosing, regardless of the duration of dose interruption.
- As with all doses of pegvaliase, an adult observer must observe the subject during study drug administration and for a minimum of 1 hour following study drug administration.

8.3.8.2 Angioedema

Angioedema is an eruption similar to urticaria but with larger, edematous wheals involving dermal, subcutaneous or submucosal tissues. It is sometimes associated with severe respiratory distress due to edema of the upper airways. Angioedema may present as a symptom (eg, lip, eye, tongue, throat, or facial swelling) of an acute systemic hypersensitivity reaction or anaphylaxis but may also present in isolation.

8.3.8.3 Serum Sickness

Serum sickness is a Type III immune hypersensitivity reaction that occurs when the immune system develops antibodies to a foreign protein. The antibody and foreign protein combine to form an immune complex that can settle in areas of the body such as the joints or the kidney.

Typical presentations include signs and symptoms commonly associated with hypersensitivity or infections: rash, itching, fever and arthralgia (MedDRA terms: serum sickness, serum sickness-like reaction and Type III immune-complex mediated reaction).

8.3.8.4 Severe Injection Site Reaction

A severe injection site reaction will be defined as any injection site reaction adverse event that is reported as CTCAE Grade 3 or above in severity and that codes to the MedDRA high level term (HLT) of Injection site reaction.

8.3.8.5 Hypophenylalaninemia

Blood Phe levels will be regularly monitored during the study. Hypophenylalaninemia is defined in this study as 2 consecutive blood Phe levels < 30 µmol/L. Hypophenylalaninemia will be managed in this study as described in Section 6.5.

8.3.8.6 Arthralgia

Arthralgia will be identified using the MedDRA PT of arthralgia and will include any synonyms of arthralgia such as musculoskeletal pain, pain in the extremity, or neck pain. Any event that meets these criteria and has a duration of > 6 months will be considered a persistent arthralgia. An arthralgia event that is reported as CTCAE Grade 3 or above in severity will be considered a severe event. Arthralgia events that require treatment with a steroid medication will also be considered an AESI.

8.3.9 Medical Device Incidents (Including Malfunctions)

Medical devices (prefilled syringes) are being provided for use in this study. In order to fulfill regulatory reporting obligations, the investigators are responsible for the detection and documentation of events meeting the definitions of device deficiency or malfunction that occur during the study with such devices.

The definition of a Medical Device deficiency can be found in Appendix 6, Section 10.6.

NOTE: Incidents fulfilling the definition of an AE/SAE will also follow the processes outlined in Section 8.3.3 and Appendix 3, Section 10.3.

8.3.9.1 Time Period for Detecting Medical Device Deficiencies

Medical device incidents or malfunctions of the device that result in an incident will be detected, documented, and reported during all periods of the study in which the medical device is used.

If the investigator learns of any device deficiency at any time after a subject has been discharged from the study, and such incident is considered reasonably related to a medical device provided for the study, the investigator will promptly notify the sponsor.

The method of documenting Medical Device deficiencies is provided in Appendix 6, Section 10.6.

8.3.9.2 Follow-up of Medical Device Deficiencies

Follow-up applies to all subjects, including those who discontinue study treatment.

The investigator is responsible for ensuring that follow-up includes any supplemental investigations as indicated to elucidate the nature and/or causality of the deficiency.

New or updated information will be recorded on the originally completed form with all changes signed and dated by the investigator.

8.3.9.3 Prompt Reporting of Device Deficiencies to Sponsor

Device deficiencies must be reported to the sponsor within 24 hours after the investigator determines that the event meets the protocol definition of a medical device deficiency.

The investigator should complete the Device Malfunction eCRF in electronic data capture (EDC). The investigator/designee must complete a Product Complaint form and submit it to the sponsor via fax or email as outlined in the Pharmacy Manual. The sponsor will be the contact for the receipt of device deficiency reports.

8.3.9.4 Regulatory Reporting Requirements for Device Deficiencies

The investigator must promptly report all device deficiencies occurring with any medical device provided for use in the study so the sponsor can fulfill their legal responsibility to notify appropriate regulatory authorities and other entities about certain safety information relating to medical devices being used in clinical studies.

The investigator, or responsible person according to local requirements (eg, the head of the medical institution), will comply with the applicable local regulatory requirements relating to the reporting of device deficiencies to the IRB/IEC.

8.4 Pharmacokinetics

Blood samples will be collected for measurement of plasma concentrations of pegvaliase as specified in the SoA (Section 1.3). For subjects randomized to active (pegvaliase) treatment, Part 1 blood samples for trough PK will be collected pre-dose (within 2 hours of dosing) at Day 1 and every 4 weeks for the first 24 weeks, followed by sampling every 8 weeks thereafter during Part 1. Intensive PK sampling will be performed at Week 73 and at dose

escalations to 20, 40, and 60 mg/day. For intensive PK, samples will be taken at pre-dose (within 2 hours of dosing) and 2, 4, 8, 12, and 24 hours post dose. The 24-hour sample will be taken prior to the next daily dose. For subjects randomized to control (diet-only) treatment, there are no PK assessments in Part 1.

For subjects randomized to active (pegvaliase) treatment, Extension Phase (Part 2) trough PK samples will be collected every 8 weeks. For subjects randomized to control (diet-only) treatment, the Part 2 PK assessment schedule will mirror the Part 1 schedule performed for active subjects, with trough PK samples collected pre-dose (within 2 hours of dosing) at Week 73 and every 4 weeks for the first 24 weeks and then every 8 weeks though the end of the study at Week 153. Intensive PK sampling will be performed Week 145 and for dose escalations to 20, 40, and 60 mg/day. For intensive PK, samples will be taken at pre-dose (within 2 hours of dosing) and 2, 4, 8, 12, and 24 hours post dose.

The PK parameters shown in Table 8.4.1 will be determined if feasible from the plasma concentration-time data for subjects who have sufficient data for PK analysis (eg, at least 3 samples with measurable concentrations during the terminal log-linear phase for estimating the terminal half-life and related parameters; at least 3 samples with measurable concentrations for other derived parameters).

Table 8.4.1: Derived Parameters for Pharmacokinetics Analysis

Parameter	Parameter Description
$AUC_{0-24hr,ss}$	Area under the plasma concentration-time curve from 0 to 24 hours at steady state, calculated using the linear trapezoidal rule
$C_{max,ss}$	Maximum observed plasma concentration within 24 hours post-dose at steady state
$T_{max,ss}$	Time to reach Cmax,ss
$C_{trough,ss}$	Trough observed plasma concentration prior to dosing at steady state
$t_{1/2}$	Terminal half-life, t1/2=ln2/λz where λz is the elimination rate constant
$CL_{ss/F}$	Apparent clearance of drug following subcutaneous injection at steady state
$V_{dz/F}$	Apparent volume of distribution based upon the terminal phase

The timing of sampling may be altered during the course of the study based on newly available data (eg, to obtain data closer to the time of peak plasma concentrations) to ensure appropriate monitoring. Instructions for the collection and handling of biological samples will be provided by the sponsor. The actual date and time (24-hour clock time) of each sample will be recorded.

Plasma samples collected for analyses of pegvaliase concentration may also be used for exploratory safety or efficacy analyses during or after the study.

Genetic analyses will not be performed on these plasma samples.

8.5 Pharmacodynamics

Blood Phe and Tyrosine (Tyr) will be measured by validated assays (see Section 8.1.1 and Section 1.3).

8.6 Biomarkers and Genetic Assessment

8.6.1 PAH Genotyping

PAH genotype testing should be performed where allowed by local health authorities and the EC/IRB.

8.6.2 Blood for Exploratory Analyses

As an exploratory analysis, blood will be collected to evaluate biochemical, molecular, and cellular aspects of PKU and to develop the assays used for these evaluations. The blood samples reserved for exploratory analyses are collected at the time points indicated in the SoA (see Section 1.3).

For each portion of blood samples reserved for protocol-specified analyses, there may be multiple sample aliquots. Once samples have been successfully analyzed during the study per protocol, unused sample portions may be used during the study and afterwards for assay development and other purposes stated in this section.

8.7 Immunogenicity Assessments

Sampling for immunogenicity testing will be performed routinely throughout the study to provide a comprehensive assessment of the immune response against pegvaliase. Antibodies against pegvaliase will be evaluated in serum samples collected from all subjects according to the SoA (Section 1.3). Additionally, serum samples should be collected at the final visit from subjects who discontinued study treatment or were withdrawn from the study.

Routine immunogenicity testing for anti-drug antibodies will be performed using validated semi-quantitative assays used in previous Phase 3 studies. Titer and incidence of positivity for each of the following analytes will be reported: total anti-pegvaliase antibodies (TAb), anti-PAL IgG, anti-PAL IgM, anti-PEG IgM, anti-PEG IgG, and pegvaliase-neutralizing antibodies (NAb). In the event of a hypersensitivity reaction visit (HRV) only, assessment of anti-pegvaliase IgE antibodies will be performed using an ImmunoCAP assay after depletion of IgG and IgM.

8.8 Health Economics/Medical Resource Utilization and Health Economics

Health Economics/Medical Resource Utilization and Health Economics parameters are not evaluated in this study.

9 STATISTICAL CONSIDERATIONS

9.1 Statistical Hypotheses

The study hypothesis is that subjects receiving pegvaliase will show a greater reduction in blood Phe following 72 weeks on study compared to the diet-only control group.

Let μ_a and μ_b denote the mean change from baseline in blood Phe following 72 weeks on study in the pegvaliase and diet-only group, respectively. The null and alternative hypotheses are:

H_0: $\mu_a = \mu_b$

H_1: $\mu_a \neq \mu_b$

The hypotheses will be tested at significance level of 0.05 (two-sided).

9.2 Sample Size Determination

Approximately 54 subjects will be enrolled into the study and randomized to the active (pegvaliase) or control (diet-only) group in 2:1 ratio. Assuming a mean (SD) reduction in Phe following 72 weeks on study of 640 (570) µmol/L in the pegvaliase group and 100 (250) µmol/L in the diet-only group, a total sample size of 54 subjects will provide more than 90% power to detect a treatment difference, based on the two-sample T-test with unequal variance and a significance level of 0.05 (two sided). The assumption of treatment effect is based on clinical data from previous pegvaliase studies and a PKU registry study. See the Statistical Analysis Plan (SAP) for additional details on the sample size calculation.

Approximately 72 subjects will be screened for study participation such that approximately 54 subjects will be enrolled in the study.

Note: Potential subjects who are screened for the purpose of determining eligibility for the study, but do not participate in the study, are not considered enrolled.

9.3 Populations for Analysis

The analysis populations are defined in Table 9.3.1.

Table 9.3.1: Analysis Populations

Population	Description
All Enrolled	All subjects (or their parent/guardians, if applicable) who agree to participate in the clinical study following completion of the informed consent process and determination of study eligibility.
ITT Population	All subjects who are randomized.
Safety Population	All subjects who received study treatment (pegvaliase or diet-only management of PKU).
Per Protocol Population	All subjects in the ITT population who are compliant with the protocol with no major protocol deviations impacting the efficacy analysis. Major protocol deviations impacting efficacy analysis will be defined and documented in the SAP.
PK Population	All subjects with at least one PK measurement

9.4 Statistical Analysis

The SAP will be finalized prior to database lock (DBL) and will include a more technical and detailed description of the statistical analyses described in this section. This section is a summary of the planned statistical analyses of the most important endpoints, including primary and key secondary endpoints.

A clinical pharmacology analysis plan will also be finalized prior to DBL.

Note: If discrepancies exist between the text of the statistical analysis as planned in the protocol, and the SAP, the SAP will prevail.

9.4.1 General Considerations

Descriptive analyses will be performed to gain an understanding of the qualitative and quantitative nature of the data collected and the characteristics of the sample collected. Continuous variables will be reported as mean, standard deviation (SD), median, minimum (min) and maximum (max). Categorical variables will be presented using absolute and relative frequency counts and percentage.

All generation of tables, listings and figures described in the SAP will be performed using SAS® version 9.4 or higher.

Full details on handling missing data and the corresponding analysis methods will be described in detail in the SAP.

9.4.2 Primary Endpoints

The primary efficacy endpoint is the change from baseline in blood Phe following 72 weeks on study. Baseline blood Phe is defined as the mean of 2 pre-treatment (Screening/Run-in)

measurements to be performed 7 to 10 days apart during the Screening/Run-in period. The blood Phe measurement following 72 weeks on study is defined as the average of the Week 69 and Week 73 blood Phe measurements.

Change from baseline will be compared between subjects in the active (pegvaliase) arm and the control (diet-only) arm. The primary efficacy endpoint will be evaluated using an Analysis of Covariance (ANCOVA) model, which will include baseline blood Phe as covariate and treatment group as factor, with missing data handled by multiple imputation method. The least square (LS) mean and 95% confidence interval will be calculated for the treatment difference between pegvaliase and diet-only arms following 72 weeks on study. Additional imputation method may be conducted as sensitivity analysis, further details will be provided in the SAP.

9.4.3 Secondary Endpoints

The secondary endpoints are as follows:

- Change from baseline in total dietary protein intake (ie, medical food and/or intact food). Baseline total protein intake is defined as the mean total dietary protein intake calculated from the 3 separate 3-day diet diaries collected during Screening/Run-in. The total dietary protein intake following 72 weeks on study is defined as the mean total dietary protein intake calculated from the diet diaries collected at the Week 69 and Week 73 visits.
- Plasma sample for trough PK

Descriptive summary will be provided. Full details will be provided in the SAP.

9.4.4 Exploratory Endpoints

Refer to the SAP for a discussion of analysis for exploratory endpoints.

9.4.5 Other Safety Analyses

Safety analyses will be performed on the Safety Population. AEs will be coded using the most recent version of the Medical Dictionary for Regulatory Activities (MedDRA). The incidence of treatment-emergent AEs, SAEs, and AESIs will be summarized by MedDRA system organ class, preferred term, relationship to study drug, and severity. Subjects with AEs that result in study drug discontinuation, dosing interruption, or dose level reduction will be tabulated. A by-subject listing of all AEs will be provided.

Clinical laboratory data will be summarized descriptively at baseline and post-baseline visits. Shift tables will be created to summarize the change in CTCAE grade from baseline to worst post-baseline value. Clinically significant laboratory abnormalities reported as AEs will be

summarized. Descriptive statistics for vital signs, physical examination results, ECG test results, and immunogenicity test results will also be provided. Details of statistical methods will be provided in the SAP.

9.5 Interim Analyses

No interim analyses are planned for this study.

9.6 Data Monitoring Committee (DMC)

The independent Data Monitoring Committee (DMC) will act in an advisory capacity to BioMarin to monitor subject safety and the efficacy of pegvaliase in subjects who participate in Study 165-306. The DMC responsibilities may include:

- Review the study protocol, informed consent and assent documents, and plans for data monitoring
- Evaluate the progress of the trial, study data quality, timeliness, subject recruitment, accrual and retention, subjects' risk versus benefit, and other factors that could affect the study outcome
- Consider relevant information that may have an impact on the safety of the subjects or the ethics of the study
- Protect the safety of the study subjects in accordance with the stopping rules as defined in study protocol
- Make recommendations to BioMarin concerning continuation, termination or other modifications of the study based on their observations of the study

Further details are provided in the DMC Charter.

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 Appendix 1: Regulatory, Ethical, and Study Oversight Considerations

10.1.1 Regulatory and Ethical Considerations

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

- Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines
- Principles of Good Clinical Practice as described in the US Code of Federal Regulations and the International Conference on Harmonisation Guidelines
- Applicable laws and regulations

The protocol, protocol amendments, ICF, Investigator Brochure, and other relevant documents (eg, advertisements) as applicable must be submitted, reviewed, and approved by the IRB/IEC before the study is initiated.

Any amendments to the protocol will require IRB/IEC approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study subjects.

The investigator will be responsible for the following:

- Providing written summaries of the status of the study to the IRB/IEC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/IEC
- Notifying the IRB/IEC of SAEs or other significant safety findings as required by IRB/IEC procedures
- Providing oversight of the conduct of the study at the site and adherence to requirements of 21 CFR, ICH guidelines, the IRB/IEC, European regulation 536/2014 for clinical studies (if applicable), and all other applicable local regulations

BioMarin will pay the full costs of the study-related tests, procedures, and treatments set forth in this protocol. In addition, after IRB/IEC approval, BioMarin may reimburse the reasonable cost of travel for study-related visits in accordance with BioMarin's travel and reimbursement policy.

The investigator should contact BioMarin immediately upon notification that a study subject has been injured by the study drug or by procedures performed as part of the study. Any subject who experiences a study-related injury should be instructed by the investigator to

seek immediate medical treatment at a pre-specified medical institution if possible, or at the closest medical treatment facility if necessary. The subject should be given the name of a person to contact to seek further information about, and assistance with, treatment for study-related injuries. BioMarin or the institution may pay for reasonable and necessary medical services to treat the injuries caused by the study drug or study procedures. In some jurisdictions, BioMarin is obligated by law to pay for study-related injuries. If this is the case, BioMarin will comply with the law. BioMarin will not pay for any hospitalizations, tests, or treatments for medical problems of any sort related solely to the study subject's primary disease or any concurrent disease and that are unrelated to the study.

Significant Noncompliance Reporting: Any deviation or series of deviations from the Protocol, ICH GCP, regulatory, and/or legal requirements that is likely to affect to a significant degree the safety or rights of a subject; the stability, quality, and/or control of study drug; or the quality or integrity of the study data shall be reported in writing to BioMarin within one (1) business day upon becoming aware of any suspicion or discovery of Significant Noncompliance.

10.1.2 Statement of Investigator and Financial Disclosure

Prior to beginning the study, the investigator at each site must provide to sponsor or designee a fully executed and signed Statement of Investigator (SOI) form. A US Food and Drug Administration (FDA) Form FDA 1572 serves as an acceptable SOI form. If Form FDA 1572 may not be used in a particular region, the investigator must provide a fully executed SOI on the form provided by the sponsor. All investigators and sub-investigators must be listed on Form FDA 1572 or its equivalent SOI. Financial Disclosure Forms must be completed with sufficient, accurate financial information as requested for all investigators and sub-investigators listed on the Form FDA 1572 or SOI who will be directly involved in the treatment or evaluation of subjects in this study. The Financial Disclosure Forms allow the sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities. Investigators must submit an updated Financial Disclosure Form to BioMarin should their response to any questions within the form or disclosure status change during the period of the study and for one year after study completion.

In accordance with FDA Form 1572 and/or principles of ICH GCP, the investigator will ensure that:

- He or 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 or she will personally conduct or supervise the study.
- He or 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 CFR Part 50 and/or ICH Sections 2.9 and 4.8 are met. As well, he or she will ensure that IRB/IEC review and approval in 21 CFR Part 56 and/or ICH Section 2.6 are met.
- He or she will report to the sponsor adverse experiences that occur in the course of the investigation in accordance with 21 CFR 312.64 and/or ICH Section 4.11.
- He or she has read and understands the information in the IB, including potential risks and side effects of the drug.
- His or her staff and all persons who assist in the conduct of the study are informed about their obligations in meeting the above commitments
- Adequate and accurate records in accordance with 21 CFR 312.62 and/or ICH Section 4.9 are kept, and those records are available for inspection in accordance with 21 CFR 312.68 and/or ICH Section 4.9.7.
- The IRB/EC/REB complies with the requirements of 21 CFR Part 56, ICH Section 3.0, and other applicable regulations, and conducts initial and ongoing reviews and approvals of the study. He or 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/REB. Additionally, he or she will not make any changes in the research without IRB/EC/REB approval, except where necessary to eliminate apparent immediate hazards to human subjects.
- He or she agrees to comply with all other requirements regarding the obligations of clinical Investigators and all other pertinent requirements in 21 CFR Part 312 and/or ICH E6 R2.

10.1.3 Informed Consent Process

The investigator or his/her representative will explain the nature of the study to the subject or his/her legally authorized representative and answer all questions regarding the study.

Subjects must be informed that their participation is voluntary. Subjects or their legally authorized representative (as determined by the laws of the jurisdiction in which the study is being conducted) will be required to sign a statement of informed consent that meets the regulatory requirements of the country or region where the subject consents, such as 21 CFR 50 (US FDA), ICH guidelines, privacy requirements including but not limited to the Health Insurance Portability and Accountability Act (HIPAA) requirements and the General Data Protection Regulation (where applicable), and the IRB/IEC or study center. If there is no applicable law addressing the issue of who may be a legally authorized representative, a legally authorized representative will be an individual recognized by institutional policy as

acceptable for providing consent on behalf of the prospective subject to the subject's participation in the procedure(s) involved in the research.

The medical record must include a statement that written informed consent was obtained before any study specific procedures were initiated and the date the written consent was obtained. The authorized person obtaining the informed consent must also sign the ICF.

Subjects must be re-consented to the most current version of the ICF(s) during their participation in the study. Subjects who reach the age of majority in their country while the study is ongoing will be asked to provide their own written consent again upon reaching the legal age of majority.

The ICF will contain a separate section that addresses the use of remaining samples for optional additional research. The investigator or authorized designee will explain to each subject the objectives of the additional research. Subjects will be told that they are free to refuse to participate and may withdraw their consent at any time and for any reason during the storage period. A separate signature will be required to document a subject's agreement to allow any remaining samples to be used for additional research.

A copy of the ICF(s) must be provided to the subject or the subject's legally authorized representative.

A subject who is rescreened is not required to sign another ICF if the rescreening occurs during the Screening/Run-in period.

10.1.4 Data Protection

Measures will be taken to ensure the privacy of subject data, including the use of subject numbers on eCRFs. A list linking subject identification numbers with subject names and other personal information will be kept in a secure place, separate from the subjects' medical records. BioMarin will follow all applicable regulations and guidelines in the relevant locality, country, and/or region to protect the privacy of subject data.

Subjects will be assigned a unique identifier by the sponsor. Any subject records or datasets that are transferred to the sponsor will contain the identifier only; subject names or any information which would make the subject identifiable will not be transferred.

The subject must be informed that his/her personal study-related data will be used by the sponsor in accordance with local data protection law. The level of disclosure must also be explained to the subject, who will be required to give consent or be given notice of the legal bases for their data to be used as described in the informed consent.

The subject must be informed that his/her medical records may be examined by authorized personnel appointed by the sponsor, by appropriate IRB/IEC members, and by inspectors from regulatory authorities.

10.1.5 Committee Structures

The following advisory and assurance activities will be implemented to support the study: monitoring committees, clinical trial disclosure, and study monitoring.

10.1.5.1 Data Monitoring Committee (DMC)

An external independent Data Monitoring Committee (DMC) will be responsible for monitoring subject safety and efficacy of pegvaliase. The DMC consisting of five members, who are each independent of the conduct of studies in the BioMarin BMN 165 (pegvaliase) development program, and are experts in the fields of PKU, clinical immunology, neuropsychology, pharmacovigilance, and biostatistics. DMC responsibilities are described in Section 9.6.

10.1.5.2 Internal Safety Data Review Board

The Internal Safety Data Review Board, consisting of a representative from BioMarin clinical sciences, safety, and biostatistics, will review all cases of potential anaphylaxis to determine which of these events meet the criteria for an acute systemic hypersensitivity reaction as summarized in Appendix 5, Section 10.5.2.

10.1.6 Dissemination of Clinical Study Data

The posting of study information and study results will comply with applicable national regulatory requirements and BioMarin's data sharing policy available at https://www.biomarin.com/data-request-form/.

10.1.7 Data Quality Assurance

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

Sites will enter study data into eCRFs in the study EDC system. Data Quality Control will be performed by BioMarin Clinical Data Management or a designee through implementation of quality control checks specified in the study data management plan and edit check specifications.

Study monitors will perform ongoing source data verification to confirm that data entered into the eCRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of subjects are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements. Qualified individuals designated by BioMarin will monitor all aspects of the study according to 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 subjects, and, if requested, agrees to assist the monitors. The investigator and staff are responsible for being present or available for consultation during routinely scheduled site visits conducted by BioMarin or its designees.

The investigator must retain all study records required by BioMarin and by the applicable regulations in a secure and safe facility. The investigator must notify BioMarin 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, that is, 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, subject 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) at least 2 years have elapsed since the formal discontinuation of clinical development of the IP. The investigator/institution should retain subject identifiers for at least 15 years after the completion or discontinuation of the study. Subject 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 a BioMarin agreement. BioMarin must be notified and will assist with retention should the investigator/institution be unable to continue maintenance of subject files for the full 15 years. It is the responsibility of BioMarin to inform the investigator/institution as to when these documents no longer need to be retained.

Representatives of BioMarin or designees may conduct an audit of a site at any time before, 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 inspection of the study. If informed of such an inspection, the investigator should notify BioMarin immediately. The investigator will ensure that the auditors/inspectors have access to the clinical supplies, site facilities, original source documentation, and all study files.

10.1.8 Source Documents

Source documents provide evidence for the existence of the subject and substantiate the integrity of the data collected. Source documents are filed at the investigator's site.

Data entered in the eCRF that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The investigator may need to request previous medical records or transfer records, depending on the study. Also, current medical records must be available.

The definition of what constitutes source data can be found in the Monitoring Plan.

10.1.9 Study and Site Start and Closure

First Act of Recruitment

The study start date is the date on which the clinical study will be open for recruitment of subjects.

The first act of recruitment is the first site open and will be the study start date.

Study/Site Termination

The sponsor reserves the right to close the site or terminate the study at any time for any reason at the sole discretion of the sponsor. Sites will be closed upon study completion. A site is considered closed when all required documents and unused study supplies have been collected and a site closure visit has been performed.

Reasons for the early closure of a site by the sponsor may include but are not limited to:

- Failure of the investigator to comply with the protocol, the requirements of the IRB/IEC or local health authorities, the sponsor's procedures, or GCP guidelines
- Inadequate recruitment of subjects by the investigator
- Discontinuation of further study treatment development

If the study is prematurely terminated or suspended, the sponsor shall promptly inform the investigators, the IECs/IRBs, the regulatory authorities, and any contract research organization(s) used in the study of the reason for termination or suspension, as specified by the applicable regulatory requirements. The investigator shall promptly inform the subject(s) and should assure appropriate subject therapy and/or follow-up.

10.1.10 Publication Policy

The results of this study may be published or presented at scientific meetings. If this is foreseen, the investigators agree to submit all manuscripts or abstracts to the sponsor at least forty-five (45) days before submission or as agreed in the Clinical Trial Agreement between BioMarin and the investigator/institution. This allows the sponsor to protect proprietary information and to provide comments.

The sponsor will comply with the requirements for publication of study results. In accordance with standard editorial and ethical practice, the sponsor will generally support publication of multicenter studies only in their entirety and not as individual site data. In this case, a coordinating investigator will be designated by mutual agreement.

BioMarin recognizes the importance of communicating medical study data and therefore encourages the publication of these data 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 may be described in the Clinical Trial Agreement between BioMarin and the investigator/institution. Consideration for authorship of all publications will be based on mutual agreement and compliance with the Uniform Requirements for Manuscripts Submitted to Biomedical Journals (“Uniform Requirements”) of the International Committee of Medical Journal Editors (ICMJE) (http://www.icmje.org/about-icmje/faqs/icmje-recommendations/) and good publication practices (GPP).

10.2 Appendix 2: Clinical Laboratory Tests

The tests detailed in Table 10.2.1 will be performed by the central laboratory.

Protocol-specific requirements for inclusion or exclusion of subjects are detailed in Section 5 of the protocol.

Additional tests may be performed at any time during the study as determined necessary by the investigator or required by local regulations.

Table 10.2.1: Clinical Laboratory Tests

Laboratory Test	Parameters		
Chemistry	Albumin Alkaline Phosphatase ALT (SGPT) AST (SGOT) Bicarbonate Direct bilirubin Total bilirubin BUN	Calcium Total cholesterol CO_2 Creatinine Creatine phosphokinase Glucose (fasting) GGT	LDH Phosphorus Potassium Total protein Sodium Uric acid
Hematology	Hemoglobin Hematocrit	WBC count RBC count	Platelet count Differential cell count
Urinalysis	Appearance Color pH Specific gravity Ketone Protein	Glucose Bilirubin Nitrite Urobilinogen Hemoglobin	Urinary albumin/creatinine ratio [a] Urinary microscopy Urinary N-methyl histamine
Pregnancy testing	Refer to Section 8.2.5		
Complement panel	C3 C4		
Other tests conducted during the study (refer to the SoA for timing)	Phenylalanine Tyrosine hs-CRP Serum tryptase level Serum cortisol [b] Erythrocyte sedimentation rate [c]		

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; C3, C4, complement components 3, 4; CO2, carbon dioxide; ESR, Erythrocyte sedimentation rate; GGT, gamma-glutamyltransferase; hs-CRP, high sensitivity C-reactive protein; LDH, lactate dehydrogenase; RBC, red blood cell; SGOT, serum glutamic-oxaloacetic transaminase; SGPT, serum glutamic-pyruvic transaminase; WBC, white blood cell.

[a] It is recommended that urine samples be obtained as a first or second morning void. Subjects with a confirmed urine/albumin creatinine ratio of ≥ 100 mg/g should be referred to a nephrologist for consultation if results were within normal range at Baseline. Subjects who had elevated results at Baseline followed by a confirmed subsequent increase of 100-200 mg/g from Baseline should also be referred to a nephrologist for consultation.

[b] Serum cortisol samples should be taken before study drug administration in the morning before 10:00 am. A home healthcare nurse may collect samples for subject convenience. If 2 results are low and abnormal, the subject will be asked to perform additional sampling for plasma ACTH and a low-dose conventional ACTH stimulation test or the ACTH test method based on the clinical practice at the site.

[c] ESR will be performed at a local laboratory.

Investigators must document their review of each laboratory safety report.

10.3 Appendix 3: Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting

10.3.1 Definition of an Adverse Event

AE Definition
• An AE is any untoward medical occurrence in a patient or clinical study subject, temporally associated with the use of study treatment, whether or not considered related to the study treatment. • NOTE: An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of study treatment.

Events Meeting the AE Definition
• Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (eg, ECG, radiological scans, vital signs measurements), including those that worsen from baseline, considered clinically significant in the medical and scientific judgment of the investigator (ie, not related to progression of underlying disease). • Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition. • New conditions detected or diagnosed after study treatment administration • Signs, symptoms, or the clinical sequelae of a suspected drug-drug interaction. • Signs, symptoms, or the clinical sequelae of a suspected overdose of either study treatment or a concomitant medication. Overdose per se will not be reported as an AE/SAE unless it is an intentional overdose taken with possible suicidal/self-harming intent. Such overdoses should be reported regardless of sequelae. • "Lack of efficacy" or "failure of expected pharmacological action" per se will not be reported as an AE or SAE. Such instances will be captured in the efficacy assessments. However, the signs, symptoms, and/or clinical sequelae resulting from lack of efficacy will be reported as AE or SAE if they fulfill the definition of an AE or SAE.

Events Not Meeting the AE Definition
• Any clinically significant abnormal laboratory findings or other abnormal safety assessments which are associated with the underlying disease, unless judged by the investigator to be more severe than expected for the subject's condition. • The disease/disorder being studied or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the subject's condition. • Medical or surgical procedure (eg, endoscopy, appendectomy): the condition that leads to the procedure is the AE. • Situations in which an untoward medical occurrence did not occur (social and/or convenience admission to a hospital).

Events Not Meeting the AE Definition
• Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen. • Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline • Planned hospitalization for childbirth (eg, scheduled Caesarean section)

10.3.2 Definition of an SAE

If an event is not an AE per the definition above, then it cannot be an SAE even if serious conditions are met (eg, hospitalization for signs/symptoms of the disease under study, death due to progression of disease).

A SAE is defined as any untoward medical occurrence that, at any dose:
a. Results in death
b. Is life-threatening The term "life-threatening" in the definition of "serious" refers to an event in which the subject was at risk of death at the time of the event. It does not refer to an event that hypothetically might have caused death if it were more severe.
c. Requires inpatient hospitalization or prolongation of existing hospitalization In general, hospitalization signifies that the subject has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious.
d. Results in persistent disability/incapacity The term disability means a substantial disruption of a person's ability to conduct normal life functions. This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (eg, sprained ankle) which may interfere with or prevent everyday life functions but do not constitute a substantial disruption.
e. Is a congenital anomaly/birth defect in the offspring of a study subject
f. Other situations/Important Medical Events: Medical or scientific judgment should be exercised in deciding whether SAE reporting is appropriate in other situations such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require medical or surgical treatment to prevent one of the other outcomes listed in the above definition. These events should usually be considered serious. Examples of such events include invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

10.3.3 Recording and Follow-Up of AEs and/or SAEs

AE and SAE Recording

- When an AE/SAE occurs, it is the responsibility of the investigator to review all documentation (eg, hospital progress notes, laboratory reports, and diagnostics reports) related to the event.
- The investigator or qualified designee will then record all relevant AE/SAE information in the eCRF.
- It is **not** acceptable for the investigator to send photocopies of the subject's medical records to BioMarin in lieu of completion of the AE/SAE eCRF page.
- There may be instances when copies of medical records for certain cases are requested by BioMarin. In these cases, all subject identifiers, with the exception of the subject number, will be redacted on the copies of the medical records before submission to BioMarin.
- The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. Whenever possible, the diagnosis (not the individual signs/symptoms) will be documented as the AE/SAE.

Assessment of Intensity

The investigator will make an assessment of intensity for each AE and SAE reported during the study and assign it to 1 of the following categories (as defined in Common Terminology Criteria for Adverse Events [CTCAE] version 5.0). The investigator should only record the single highest grade of intensity for each AE and SAE in the eCRF. Adverse events that do not have a corresponding CTCAE term will be assessed according to the general guidelines for grading:

- Grade 1 (Mild): asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated
- Grade 2: (Moderate): minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL) (eg, preparing meals, shopping for groceries or clothes, using the telephone, managing money)
- Grade 3 (Severe): medically significant but not immediately life-threatening: hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL (eg, bathing, dressing/undressing, feeding oneself, using the toilet, taking medications, not bedridden)
- Grade 4: Life threatening consequences; urgent intervention indicated
- Grade 5: Death related to AE

An event is defined as "serious" when it meets at least 1 of the predefined outcomes as described in the definition of an SAE, NOT when it is rated as severe.

Assessment of Causality

- The investigator is obligated to assess the relationship between study treatment and each occurrence of each AE/SAE.
- An assessment of "related" means that there are facts, evidence, and/or arguments to suggest a causal relationship such that a relationship cannot be ruled out.
- The investigator will use clinical judgment to determine the relationship.

Assessment of Causality
• Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to study treatment administration will be considered and investigated. • For each AE/SAE, the investigator must document in the medical notes that he/she has reviewed the AE/SAE and has provided an assessment of causality. • There may be situations in which an SAE has occurred, and the investigator has minimal information to include in the initial report to BioMarin. However, it is very important that the investigator always make an assessment of causality for every event before the initial transmission of the SAE data to BioMarin. • The investigator may change his/her opinion of causality in light of follow-up information and send a SAE follow-up report with the updated causality assessment.

Follow-up of AEs and SAEs
• The investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by BioMarin to elucidate the nature and/or causality of the AE or SAE as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals. • If a subject dies during participation in the study or during a recognized follow-up period, the investigator will provide a copy of any post-mortem findings including histopathology. • New or updated information will be recorded in the originally completed eCRF. • The investigator will submit any updated SAE data to the sponsor within 24 hours of receipt of the information.

10.3.4 Reporting of SAEs and Events of Special Interest

SAE/AESI Reporting to BioMarin Pharmacovigilance (BPV) via an Electronic Data Collection Tool
• The primary mechanism for reporting an SAE/AESI to BPV will be the electronic data collection tool. AESI reporting will follow the SAE reporting requirements. • If the electronic system is unavailable, then the site will use the paper SAE data collection tool (see next section) in order to report the event within 24 hours. • The site will enter the SAE/AESI data into the electronic system as soon as it becomes available. • After the study is completed at a given site, the electronic data collection tool will be taken off-line to prevent the entry of new data or changes to existing data. • If a site receives a report of a new SAE/AESI from a study subject or receives updated data on a previously reported SAE/AESI after the electronic data collection tool has been taken off-line, then the site can report this information on a paper SAE form (see next section) or to BPV by telephone.

SAE/AESI Reporting to BPV via Paper CRF
• Facsimile transmission or email of the paper CRF (pregnancy form, pregnancy outcome, or SAE form is EDC unavailable) is the preferred method to transmit this information to BPV. • In rare circumstances and in the absence of facsimile equipment, notification by telephone is acceptable with a copy of the paper CRF sent by overnight mail or courier service. • Initial notification via telephone does not replace the need for the investigator to complete and sign the CRF pages within the designated reporting time frames.

Contact information for BioMarin Pharmacovigilance is as follows:

BioMarin Pharmaceutical Inc.
Address 105 Digital Drive
Novato, CA 94949
Phone: PI
Fax:
E-mail: drugsafety@bmrn.com

The Investigator is encouraged to discuss with the medical monitor any AEs for which the issue of seriousness is unclear or questioned. Contact information for the medical monitor is provided on the signature page at the front of this protocol.

Name: PI, RN, MS
Address: 105 Digital Drive
Novato, CA 94949 USA
Phone: PI
Fax:
E-mail:

10.4 Appendix 4: Contraceptive and Barrier Guidance

Definitions:

Woman of Childbearing Potential (WOCBP)

A woman is considered fertile following menarche and until becoming post-menopausal unless permanently sterile (see below).

If fertility is unclear (eg, amenorrhea in adolescents or athletes) and a menstrual cycle cannot be confirmed before first dose of study treatment, additional evaluation should be considered.

Women in the following categories are not considered WOCBP:

- Premenarchal
- Premenopausal female with 1 of the following:
    - Documented hysterectomy
    - Documented bilateral salpingectomy
    - Documented bilateral oophorectomy

  For individuals with permanent infertility due to a medical cause other than the above (eg, mullerian agenesis, androgen insensitivity), investigator discretion should be applied in determining study eligibility.

  Note: Documentation can come from the site personnel's review of the subject's medical records, medical examination, or medical history interview.
- Postmenopausal female
    - A postmenopausal state is defined as no menses for 12 months without an alternative medical cause.
        - A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a postmenopausal state in women not using hormonal contraception or hormonal replacement therapy (HRT). However, in the absence of 12 months of amenorrhea, confirmation with more than one FSH measurement is required.
    - Females on HRT whose menopausal status is in doubt will be required to use one of the non-estrogen hormonal highly effective contraception methods if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of postmenopausal status before study enrollment.

Contraception Guidance:

If sexually active, must be willing to use two acceptable methods of contraception while participating in the study and 4 weeks after the last dose of pegvaliase.

10.5 Appendix 5: Anaphylaxis Criteria

For the purposes of this study, the term anaphylaxis refers to the important identified risk of acute systemic hypersensitivity reaction. An acute systemic hypersensitivity reaction is one that is consistent with the NIAID/FAAN definition as assessed by BioMarin using the criteria listed below in Appendix 5, Section 10.5.1 and Appendix 5, Section 10.5.2.

10.5.1 Sampson's Anaphylaxis Criteria

According to the National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network (NIAID/FAAN) Second Symposium on the definition and management of anaphylaxis, anaphylaxis is highly likely when any one of the following 3 criteria are fulfilled:

1. Acute onset of an illness (minutes to several hours) with involvement of the skin, mucosal tissue, or both (eg, generalized hives, pruritus or flushing, swollen lips-tongue-uvula)

   AND AT LEAST ONE OF THE FOLLOWING:

   a. Respiratory compromise (eg, dyspnea, wheeze-bronchospasm, stridor, reduced peak expiratory flow [PEF], hypoxemia)

   b. Reduced blood pressure (BP) or associated symptoms of end-organ dysfunction (eg, hypotonia [collapse], syncope, incontinence)

2. Two or more of the following that occur rapidly after exposure *to a likely allergen for that patient* (minutes to several hours):

   a. Involvement of the skin-mucosal tissue (eg, generalized hives, itch-flush, swollen lips-tongue-uvula)

   b. Respiratory compromise (eg, dyspnea, wheeze-bronchospasm, stridor, reduced PEF, hypoxemia)

   c. Reduced BP or associated symptoms (eg, hypotonia [collapse], syncope, incontinence)

   d. Persistent gastrointestinal symptoms (eg, crampy abdominal pain, vomiting)

3. Reduced BP after exposure to *known allergen for that patient* (minutes to several hours):

a. Infants and children: low systolic blood pressure (age specific) or greater than 30% decrease is systolic BP

b. Adults: systolic BP of less than 90 mmHg or greater than 30% decrease from that person's baseline.

Source: Sampson 2006.

10.5.2 Acute Systemic Hypersensitivity Reaction

An acute systemic hypersensitivity reaction is a Type III acute systemic allergic reaction. An acute systemic hypersensitivity reaction has a similar clinical presentation to an anaphylactic reaction, but only meets the definition if the italicized criteria below are met in addition to the criteria outlined in Sampson's criteria (see Appendix 5, Section 10.5.1, Sampson's Criteria):

Sampson Criteria Number	Sampson Criteria Wording With Additional Criteria Necessary to Meet Acute Systemic Hypersensitivity Reaction Criteria Shown in Italics
1	Acute onset of an illness (minutes to several hours) with involvement of the skin, mucosal tissue, or both (eg, generalized hives, pruritus or flushing, swollen lips-tongue-uvula). *Must be generalized skin reaction to meet criteria*.
1a	Respiratory compromise (eg, dyspnea, wheeze-bronchospasm, stridor, reduced PEF, hypoxemia). *Respiratory compromise is demonstrated by oxygen desaturation below 88% on room air.*
1b	Reduced BP or associated symptoms of end-organ dysfunction (eg, hypotonia [collapse], syncope, incontinence). *Systolic BP less than 90 mmHg (adults) or 70 mmHg (children) or greater than 30% decrease from baseline*.
2d	Persistent gastrointestinal symptoms (eg, crampy abdominal pain, vomiting). *Gastrointestinal symptoms are persistent with multiple episodes that continue for several hours or more*.

10.5.3 Anaphylaxis Analysis

During the initial BLA for Palynziq in the US, the FDA proposed that anaphylaxis should be defined for labelling purposes using the methodology developed by FDA's Division of Pulmonary, Allergy, and Rheumatology Products (DPARP). As a result, in the study analyses for 165-306 anaphylaxis, including derivation of the incidence and event rates, data will be presented using both BioMarin's definition of an acute systemic hypersensitivity reaction described above and the FDA's assessment methodology, ie, a case will be counted as an anaphylaxis event if it meets any of the following criteria:

- Clinical manifestations consistent with NIAID/FAAN criterion #1 (skin involvement + respiratory or cardiovascular compromise); or

- Verbatim reports of anaphylaxis/anaphylactic reaction (or synonyms), even if NIAID/FAAN criteria were not met; or
- Cases involving subject injection of epinephrine alone, even if the subject did not experience signs/symptoms necessary to meet NIAID/FAAN criteria.

Further details are provided in the SAP.

10.6 Appendix 6: Medical Device Adverse Events, Adverse Device Effects (ADEs), Serious Adverse Events (SAEs), and Device Deficiencies: Definition and Procedures for Recording, Evaluating, Follow-Up, and Reporting

Medical Device Adverse Event reporting applies only to US sites. The definitions and procedures detailed in this appendix are in accordance with ISO 14155.

Both the investigator and the sponsor will comply with all local medical device reporting requirements.

The detection and documentation procedures described in this protocol apply to all sponsor medical devices provided for use in the study. For this study, prefilled syringes are provided (see Section 6.1.1).

10.6.1 Definition of AE and ADE

AE and ADE Definition

- An AE is defined as any untoward medical occurrence, unintended disease or injury, or untoward clinical signs (including abnormal laboratory finding) in study subjects, users, or other persons, whether or not related to the investigational medical device. This definition includes events related to the investigational medical device or comparator and events related to the procedures involved except for events in users or other persons, which only include events related to investigational devices.
- An adverse device effect (ADE) is defined as an adverse event related to the use of an investigational medical device. This definition includes any adverse events resulting from insufficient or inadequate instructions for use, deployment, implantation, installation, or operation, or any malfunction of the investigational medical device as well as any event resulting from use error or from intentional misuse of the investigational medical device.

10.6.2 Definition of SAE, SADE, and USADE

If an event is not an AE per definition above, then it cannot be an SAE even if serious conditions are met (eg, hospitalization for signs/symptoms of the disease under study, death due to disease progression).

An SAE is an AE that:

- Led to death
- Led to serious deterioration in the health of the subject resulting in either:
  1. A life-threatening illness or injury. The term 'life-threatening' in the definition of 'serious' refers to an event in which the subject was at risk of death at the time of the event. It does not refer to an event which hypothetically might have caused death if it were more severe.
  2. A permanent impairment of a body structure or a body function
  3. Inpatient or prolonged hospitalization, planned hospitalization for a pre-existing condition, or a procedure required by the protocol, without serious deterioration in health, is not considered an SAE.
  4. Medical or surgical intervention to prevent life-threatening illness or injury or permanent impairment to a body structure or a body function
- Led to fetal distress, fetal death, or a congenital abnormality or birth defect

SADE definition:

- A SADE is defined as an adverse device effect that has resulted in any of the consequences characteristic of a serious adverse event.

USADE definition

- A USADE is a serious adverse device effect which by its nature, incidence, severity, or outcome has not been identified in the current version of the risk analysis report.

10.6.3 Definition of Device Deficiency

Device deficiency definition

- A device deficiency is an inadequacy of a medical device with respect to its identity, quality, durability, reliability, safety, or performance. Device deficiencies include malfunctions, use errors, and inadequate labeling.

10.6.4 Recording and Follow-Up of AE and/or SAE and Device Deficiencies

AE, SAE, and Device Deficiency Recording

- When an AE/SAE/device deficiency occurs, it is the responsibility of the investigator to review all documentation (e.g., hospital progress notes, laboratory reports, and diagnostics reports) related to the event.
- The investigator will then record all relevant AE/SAE/device deficiency information in the subject's medical records, in accordance with the investigator's normal clinical practice and on the appropriate form of the eCRF.
- All ADEs will be entered into the AE eCRF.
- SADEs will be entered into the AE eCRF and submitted to BPV. The site will also complete and submit the study specific paper device report form within 24 hours of site awareness.
- It is not acceptable for the investigator to send photocopies of the subject's medical records to BPV in lieu of completion of the /AE/SAE/device deficiency eCRF page.
- There may be instances when copies of medical records for certain cases are requested by BPV. In this case, all subject identifiers, with the exception of the subject number, will be redacted on the copies of the medical records before submission to BPV.
- The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. Whenever possible, the diagnosis (not the individual signs/symptoms) will be documented as the AE/SAE.
- For device deficiencies, it is very important that the investigator describes any corrective or remedial actions taken to prevent recurrence of the incident.
- A remedial action is any action other than routine maintenance or servicing of a medical device where such action is necessary to prevent recurrence of a device deficiency. This includes any amendment to the device design to prevent recurrence.

Assessment of Intensity

- The investigator will make an assessment of intensity for each AE/SAE/device deficiency reported during the study and assign it to one of the following categories:
  - Mild: An event that is easily tolerated by the subject, causing minimal discomfort and not interfering with everyday activities.
  - Moderate: An event that causes sufficient discomfort and interferes with normal everyday activities.
  - Severe: An event that prevents normal everyday activities. An AE that is assessed as severe should not be confused with a SAE. Severe is a category utilized for rating the intensity of an event; and both AEs and SAEs can be assessed as severe.
- An event is defined as 'serious' when it meets at least 1 of the predefined outcomes as described in the definition of an SAE, NOT when it is rated as severe.

Assessment of Causality

- The investigator is obligated to assess the relationship between study treatment and each occurrence of each AE/SAE/device deficiency.
- A "reasonable possibility" of a relationship conveys that there are facts, evidence, and/or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out.
- The investigator will use clinical judgment to determine the relationship.
- Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to study treatment administration will be considered and investigated.
- The investigator will also consult the IB in his/her assessment.
- For each AE/SAE/device deficiency, the investigator must document in the medical notes that he/she has reviewed the AE/SAE/device deficiency and has provided an assessment of causality.
- There may be situations in which an SAE has occurred and the investigator has minimal information to include in the initial report to BPV. However, it is very important that the investigator always make an assessment of causality for every event before the initial transmission of the SAE data to BPV.
- The investigator may change his/her opinion of causality in light of follow-up information and send a SAE follow-up report with the updated causality assessment.
- The causality assessment is one of the criteria used when determining regulatory reporting requirements.

Follow-up of AE/SAE/device deficiency

- The investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by BPV to elucidate the nature and/or causality of the AE/SAE/device deficiency as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.
- If a subject dies during participation in the study or during a recognized follow-up period, the investigator will provide BPV with a copy of any post mortem findings including histopathology.
- New or updated information will be recorded in the originally completed eCRF.
- The investigator will submit any updated SAE data to BPV within 24 hours of receipt of the information.

10.6.5 Reporting of SAEs

SAE Reporting to BPV via an Electronic Data Collection Tool

- The primary mechanism for reporting an SAE to BPV will be the electronic data collection tool.
- If the electronic system is unavailable, then the site will use the paper SAE data collection tool (see next section) in order to report the event within 24 hours.
- The site will enter the SAE data into the electronic system as soon as it becomes available.
- After the study is completed at a given site, the electronic data collection tool will be taken off-line to prevent the entry of new data or changes to existing data.
- If a site receives a report of a new SAE from a study subject or receives updated data on a previously reported SAE after the electronic data collection tool has been taken off-line, then the site can report this information on a paper SAE form (see next section) or to BPV by telephone.
- Contacts for SAE reporting can be found in Appendix 3, Section 10.3.4.

SAE Reporting to BPV via Paper CRF

- Facsimile transmission of the SAE paper CRF is the preferred method to transmit this information to BPV.
- In rare circumstances and in the absence of facsimile equipment, notification by telephone is acceptable with a copy of the SAE data collection tool sent by overnight mail or courier service.
- Initial notification via telephone does not replace the need for the investigator to complete and sign the SAE CRF pages within the designated reporting time frames.
- Contacts for SAE reporting can be found in Appendix 3, Section 10.3.4.

10.6.6 Reporting of SADEs

SAE Reporting to BPV

NOTE: There are additional reporting obligations for medical device incidents that are potentially related to SAEs that must fulfil the legal responsibility to notify appropriate regulatory authorities and other entities about certain safety information relating to medical devices being used in clinical studies.

- Any device deficiency that is associated with an SAE must be reported to the sponsor within 24 hours after the investigator determines that the event meets the definition of a device deficiency.
- The sponsor shall review all device deficiencies and determine and document in writing whether they could have led to an SAE. These shall be reported to the regulatory authorities and IRBs/IECs as required by national regulations.
- Contacts for SAE reporting can be found in Appendix 3, Section 10.3.4.

10.7 Appendix 7: Abbreviations

Abbreviation	Definition
ACMG	American College of Medical Genetics and Genomics
ADHD	Attention Deficit Hyperactivity Disorder
ADHD-RS	Attention Deficit Hyperactivity Disorder Rating Scale
ADL	activities of daily living
AE	adverse event
AESI	adverse events of special interest
ALT	alanine aminotransferase
ANCOVA	Analysis of Covariance
AST	aspartate aminotransferase
AUC	area under the concentration-time curve
BH4	tetrahydrobiopterin
BLA	Biologics License Application
BPV	BioMarin Pharmacovigilance
BRIEF	Behavior Rating Inventory of Executive Function
C3	complement component 3
C4	complement component 4
CIOMS	Council for International Organizations of Medical Sciences
C_{max}	maximum observed plasma concentration
CONSORT	Consolidated Standards of Reporting Trials
CRF	case report form
CRP	C-reactive protein
CTCAE	Common Terminology Criteria for Adverse Events
DBL	database lock
DFRF	Directed Follow-up Report Form
DMC	Data Monitoring Committee
DPARP	FDA Division of Pulmonary, Allergy, and Rheumatology Products
DRE	disease-related event
DRI	Dietary Reference Intake
DSM	Diagnostic and Statistical Manual of Mental Disorders
ECG	electrocardiogram
EC/IRB	Ethics Committee/Institutional Review Board
eCRF	electronic Case Report Form
EDC	electronic data capture
ET	Early Termination

Abbreviation	Definition
EU	European Union
FDA	Food and Drug Administration
FSH	follicle stimulating hormone
GCP	good clinical practice
GPP	good publication practice
HAE	hypersensitivity adverse event
HIPAA	Health Insurance Portability and Accountability Act
HPA	hyperphenylalaninemia
HRT	hormonal replacement therapy
HRV	hypersensitivity reaction visit
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICMJE	International Committee of Medical Journal Editors
IgE	immunoglobulin E
IgG	immunoglobulin G
IgM	immunoglobulin M
IMP	investigational medical products
IND	investigational new drug
IP	investigational product
IQ	intelligence quotient
ISR	injection site reaction
I/T/M	Induction/Titration/Maintenance (dosing regimen)
LS	least square
MedDRA	Medical Dictionary for Regulatory Activities
MNT	medical nutritional therapy
NAb	neutralizing antibody
NCI-CTCAE	National Cancer Institute – Common Terminology Criteria for Adverse Events
NIAID/FAAN	National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network
NIMP	non-investigational medical products
NPKUA	National PKU Alliance
NSAID	nonsteroidal anti-inflammatory drug
PAH	phenylalanine hydroxylase
PAL	phenylalanine ammonia lyase
PDCO	European Medicines Agency's Paediatric Committee
PEG	polyethylene glycol

Abbreviation	Definition
PFS	pre-filled syringe
Phe	phenylalanine
PIP	Paediatric Investigational Plan
PK	pharmacokinetics
PKU	phenylketonuria
PRN	pro re nata (as needed)
rAvPAL-PEG	PEGylated recombinant Anabaena variabilis phenylalanine ammonia lyase
RCT	randomized control trial
RDI	recommended daily intake
REMS	Risk Evaluation and Mitigation Strategy
SAE	serious adverse event
SAP	Statistical Analysis Plan
sBLA	supplemental Biologics License Application
SC	Study Completion
SD	standard deviation
SoA	Schedule of Activities
SOI	Statement of Investigator
TAb	total anti-drug antibody
Tyr	tyrosine
ULN	upper limit of normal
US	United States
WOCBP	Woman of Childbearing Potential

10.8 Appendix 8: Protocol Amendment History

The Protocol Amendment Summary of Changes Table for the current amendment is located directly before the Table of Contents (TOC). This is the first amendment to this protocol.

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CLINICAL STUDY PROTOCOL

Protocol Title:	A Phase 3 Multi-Center Study to Evaluate the Safety and Efficacy of Subcutaneous Injections of Pegvaliase in Adolescent Subjects (Ages 12-17) with Phenylketonuria Featuring an Open-Label Randomized Two-Arm (Active vs Diet-Only Control) Design
Protocol Number:	165-306
Amendment Number:	Amendment 2
Compound:	pegvaliase (formerly BMN 165)
Short Title:	A Phase 3 Multi-Center Study to Evaluate the Safety and Efficacy of Subcutaneous Injections of Pegvaliase in Adolescent Subjects (Ages 12-17) with Phenylketonuria
Study Phase:	Phase 3
Sponsor Name:	BioMarin Pharmaceutical Inc.
Legal Registered Address:	105 Digital Drive Novato, CA 94949
Regulatory Agency Identifier Number(s):	IND: 076269 EudraCT: 2021-005058-27
Amendment 1 Date:	26 January 2022
Amendment 2 Date:	23 October 2023

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May not be divulged, published, or otherwise disclosed to others without prior written approval from BioMarin.
This study will be conducted according to the principles of Good Clinical Practice as described in the U.S. Code of Federal Regulations and the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use guidelines, including the archiving of essential documents.

I have read this study protocol and agree to conduct this study in compliance with the protocol, all applicable regulations, guidelines, and other information supplied to me.

Investigator Signature Date

Printed name: _____

Accepted for the Sponsor:

A proof of electronic signature page is appended to the back of this protocol

Medical Monitor Signature Date

Printed name: PI , RN, MS PI Clinical Science

Medical monitor contact information is available in Appendix 3, Section 10.3.

PROTOCOL AMENDMENT SUMMARY OF CHANGES TABLE

DOCUMENT HISTORY	
Document	**Approval date**
Amendment 2	23 October 2023
Amendment 1	26 January 2022
Original Protocol	22 September 2021

Amendment 2 (23 October 2023):

Overall Rationale for the Amendment

The protocol is being amended to include clinical outcome assessments (COAs) and to capture various administrative changes to further clarify some aspects of the protocol noted since the implementation of Amendment 1. Additionally, minor typographic and consistency errors are also being corrected.

Section Number and Name	Description of Change	Brief Rationale
1.1 Synopsis 1.3 Schedules of Activities Table 3.1 Objectives and Endpoints 8.1.3.1 Neurocognitive Assessments 8.1.3.2 Global Impression Scales 11 References	Added clinical outcome assessment (COA) exploratory endpoints: PKU-SSIS v2.0 (adolescent version) and accompanying global items (Patient Global Impression of Severity/Change [PGIS/C], Caregiver Global Impression of Severity/Change [CaGIS/C], and Clinician Global impression of Severity [CGIS].	The PKU-SSIS v2.0 (adolescent version) is a newly developed PKU-specific patient-reported outcome (PRO) measure to assess symptoms and impacts of PKU in adolescents with PKU. Global impression items are included to support validation of the PKU-SSIS in this patient population.
Table 2.3.1.1 Risks Associated with Study Drug 6.5 Dose Modification	Clarified that a pegvaliase dose reduction is required in the event of hypophenylalaninemia.	Previous language proposed that either dose reduction or dietary protein changes were acceptable risk mitigation for normalization of hypophenylalaninemia. Dose reduction must be performed, and a dietary protein change is an additional option for managing hypophenylalaninemia
1.3 Schedules of Activities 8.4 Pharmacokinetics	Added PK collection sample time windows.	To allow flexibility for specimen collection times.

Section Number and Name	Description of Change	Brief Rationale
1.1 Synopsis Figure 1.2.2 Dosing Schematic 1.3 Schedules of Activities 4.1 Overall Design 6.5 Dose Modification 8 Study Assessments and Procedures	Duration of Part 2 of the study extended from 80 weeks to 142 weeks (total study duration 214 weeks)	Duration of Part 2 extended due to longer enrollment period
1.1 Synopsis Table 6.1.1 Study Treatment 6.5 Dose Modification Table 6.5.1 Study Drug Dose Titration Schedule	Include additional option of 5 mg dose level during induction/titration	Provide investigators with discretion to use an additional dosing option during induction/titration
1.3 Schedules of Activities Table 10.2.1 Clinical Laboratory Tests	Urine N-methylhistamine test added to Day 1 and hypersensitivity visit in the Schedules of Activities	Added to clarify discrepancy within protocol
Figure 1.2.2 Dosing Schematic 6.5 Dose Modification Table 6.5.1 Study Drug Dose Titration Schedule	Previous requirement for blood Phe to be ≤ 360 µmol/L for dose escalations has been changed from a requirement to a recommendation	To be consistent with investigator discretion
1.1 Synopsis 1.3 Schedules of Activities Table 3.1 Objectives and Endpoints 8.1.2 Total Dietary Protein Intake, Dietary Monitoring, and Dietary Protocol Restrictions 9.4.3 Secondary Endpoints	Total dietary protein should be measured in grams per kilogram (g/kg)	Clarified total dietary protein unit

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1 PROTOCOL SUMMARY

1.1 Synopsis

Protocol Title: A Phase 3 Multi-Center Study to Evaluate the Safety and Efficacy of Subcutaneous Injections of Pegvaliase in Adolescent Subjects (Ages 12-17) with Phenylketonuria Featuring an Open-Label Randomized Two-Arm (Active vs Diet-Only Control) Design

Short Title: A Phase 3 Multi-Center Study to Evaluate the Safety and Efficacy of Subcutaneous Injections of Pegvaliase in Adolescent Subjects (Ages 12-17) with Phenylketonuria

Study Design Rationale:

This Phase 3 multicenter study is designed to evaluate the safety and efficacy of pegvaliase administered daily to adolescent participants (ages 12 to 17 years old (US), inclusive, and 12 to 15 years old (EU), inclusive) with phenylketonuria (PKU). Participants will be randomized in a 2:1 ratio to the active (pegvaliase) and control (diet-only) treatment arms, respectively, with 36 participants receiving pegvaliase and 18 participants managing their PKU with diet alone. The primary efficacy endpoint is the change from baseline in blood phenylalanine (Phe) following 72 weeks on study (the end of Part 1). Beginning Week 73, participants in the active (pegvaliase) treatment arm will continue to receive pegvaliase in the Extension Phase (Part 2) for up to an additional 142 weeks (through Week 215). Participants in the control (diet only) treatment arm will initiate pegvaliase treatment beginning Week 73 and, from Weeks 73 through 215, follow the same dosing and assessment schedule that the active participants followed.

A randomized control trial (RCT) design, with diet-only management of PKU as the control, will allow for robust data collection and minimize the risk of confounding factors influencing the results. An open-label diet-only control arm is the best comparator for this age group as daily placebo injections would place undue burden on adolescent PKU patients.

In Part 1 (through the Week 73 visit), randomized participants in both the control (diet-only) and active (pegvaliase) treatment arms will be required to maintain the same level of protein intake they established during Screening/Run-in. In Part 2, participants in the control (diet-only) arm must continue to maintain this prescribed level of protein intake, whereas participants randomized to the active (pegvaliase) arm whose blood Phe concentration is ≤ 360 µmol/L for a minimum of 4 weeks may adjust dietary protein intake based on individual participant response to pegvaliase and guidance from the investigator. Participants

are required to maintain a consistent level of protein intake during the study to ensure that the safety and efficacy endpoints are attributable to pegvaliase rather than to changes in dietary protein intake.

Pegvaliase will be subcutaneously injected in a hospital setting for the first 8 weeks and thereafter at the clinic or at home by a trained participant, caregiver, adult observer, or visiting healthcare provider. Pegvaliase will also be injected in a hospital setting for any subsequent dose escalations. Administration at home makes daily treatment less burdensome for participants, thereby improving treatment compliance.

An adult observer is required for a minimum of 1 hour post injection throughout the study. An adult observer is defined as someone who will be present with the participant during and after pegvaliase administration, is able to recognize the signs and symptoms of an anaphylaxis episode, and is able to call for emergency medical support and administer epinephrine, if warranted. The requirement for an adult observer is included to ensure that participants self-administering pegvaliase would have support in recognizing and quickly responding to signs and symptoms of anaphylaxis.

The primary efficacy endpoint is the change from treatment naïve baseline in blood Phe following 72 weeks on study (the end of Part 1). Blood Phe level is the established clinical marker used to diagnose PKU and assess disease control, and reduction of blood Phe concentration to target levels is the universally acknowledged treatment goal for PKU patients.

Objectives and Endpoints:

The primary, secondary, and exploratory objectives and associated endpoints are shown below. Refer to the Schedule of Activities (SoA) for the complete listing of assessment timepoints.

Objectives	Endpoints
Primary	
To evaluate the efficacy of pegvaliase in adolescent participants with PKU	The primary efficacy endpoint is change in blood Phe concentration from treatment naïve baseline following 72 weeks on study. Change in blood Phe from baseline is compared between participants in the active (pegvaliase) and the control (diet-only) treatment arms.
To evaluate the safety of pegvaliase in adolescent participants with PKU	• Adverse events (AEs), including serious AEs (SAEs) • Clinical laboratory tests (chemistry, hematology, and urinalysis) results • Vital signs • Physical examination (including height, weight) • Electrocardiogram (ECG) test results • Immunogenicity test results (anti-PEG IgG, anti-PEG IgM, anti-PAL IgG, anti-PAL IgM, Tab, Nab, and anti-pegvaliase IgE [hypersensitivity reaction visits only]).
Secondary	
To characterize total dietary protein intake in adolescent participants with PKU after pegvaliase treatment	Total dietary protein intake must remain stable for change in blood Phe, the primary endpoint, to be accurately assessed. To gauge the stability of total protein intake, the change from baseline at Week 73 in total dietary protein intake (measured in grams/kilogram [g/kg]) (defined as the sum of the protein intake from medical food and intact food) will be measured.
To characterize the pharmacokinetics (PK) of pegvaliase in adolescent participants with PKU	Trough plasma PK samples will be collected and analyzed as specified in the SoA. PK parameters assessed with intensive PK sampling will include $AUC_{0\text{-}24hr,ss}$, $C_{max,ss}$, $T_{max,ss}$, $t_{1/2}$, $CL_{ss/F}$, and $V_{dz/F}$. For participants randomized to the active (pegvaliase) treatment arm, intensive PK sampling will be performed at Week 73 and for dose escalations to 20, 40, and 60 mg/day. For participants randomized to the control (diet-only) treatment arm, there are no PK assessments in Part 1. In Part 2, intensive PK sampling will be performed at Week 145 and for dose escalations to 20, 40, and 60 mg/day.

Objectives	Endpoints
Exploratory Objectives	
To evaluate the effect of pegvaliase treatment on neurocognitive outcomes in adolescent participants with PKU.	• The ADHD Rating Scale IV inattention subscale (ADHD RS-IV) rated by the parent/guardian and performed at Baseline (Day 1, pre-dose) and every 12 weeks throughout the study. • The Behavior Rating Inventory of Executive Function, Second Edition (BRIEF2) rated by the parent/guardian and performed at Baseline (Day 1, pre-dose) and every 12 weeks throughout the study. • Change from baseline (or first administration) in Phenylketonuria Symptom Severity and Impacts Scale (PKU-SSIS) Version 2.0 scores.
To assess changes in PKU symptoms and impacts	• Change from baseline (or first administration) in global severity items (Patient Global Impression of Severity [PGIS], Caregiver Global Impression of Severity [CaGIS], and Clinician Global Impression of Severity [CGIS]). • Change in global change items (Patient Global Impression of Change [PGIC] and Caregiver Global Impression of Change [CaGIC]).
To compare participants' optimal maintenance pegvaliase dose across different age groups within the study	• Time to participants' optimal maintenance dose compared to older age subsets • Number of participants who require the highest (60 mg/day) maintenance dose • Percentage/number of participants in the blood Phe target ranges of < 600 µmol/L and < 360 µmol/L.
Explore the biochemical, molecular, and cellular aspects of PKU	Blood will be collected to evaluate biochemical, molecular, and cellular aspects of PKU and to develop the assays used for these evaluations.
PAH genotyping	Testing will be performed on samples collected Day 1 pre-dose where allowed by local health authorities and the EC/IRB.

Overall Design:

Study 165-306 is an open-label, two-arm, randomized control evaluation of pegvaliase in approximately 54 participants (ages 12 to 17 years old (US), inclusive, and 12 to 15 years old (EU), inclusive) with diet only as the control arm. To be eligible for the study, potential participants must meet all eligibility requirements described in the protocol, including the following selected key inclusion criteria:

- Is 12 to 17 years old (US), inclusive, or 12 to 15 years old (EU), inclusive, at the start of the Screening/Run-in Period (Day 28).

- Diagnosis of PKU and failure to maintain recommended blood Phe levels on existing management (defined as having failed sapropterin dihydrochloride treatment and Phe-restricted diet) demonstrated by 2 blood Phe concentration measurements > 600 µmol/L during the Screening/Run-in Period (7 to 10 days between blood Phe assessments) and average blood Phe concentration > 600 µmol/L over the past 12 months (per available data).
- Willing and able to maintain and adjust dietary and medical protein food intake according to the study protocol and under the supervision of study dietician or adequately trained designee per investigator during study participation.
- An adult (≥ 18 years of age) has been identified who is willing and competent to observe the participant during study drug administration and for a minimum of 1 hour following administration. A home healthcare nurse may perform the study drug observations consistent with local regulatory and site requirements.

Selected key exclusion criteria include the following:

- Potential participants are not eligible if they are unable to identify and/or communicate to others that they are experiencing symptoms of potential anaphylaxis due to cognitive impairment or other reasons.

Following enrollment, participants will be randomized 2:1 to the active (pegvaliase) and control (diet-only) treatment arms, with 36 participants receiving daily pegvaliase and 18 participants managing PKU by diet alone. Treatment assignment will be stratified by Screening/Run-In blood Phe (mean of the 2 pre-treatment blood Phe assessments performed during the Screening/Run-in period) of ≤ 1000 µmol/L or > 1000 µmol/L. The primary efficacy endpoint is the change from baseline blood Phe following 72 weeks on study (the end of Part 1). Participants in the active and control arms will follow the same visit schedules and perform the same assessments throughout the 72-week Primary Treatment Phase (Part 1) except that the control participants will not be receiving pegvaliase so will not have PK draws or immunogenicity assessments.

After providing informed consent, participants will enter the Screening/Run-in period, which will last 4 weeks and include 2 assessments of blood Phe levels, 7 to 10 days apart, to determine eligibility. Following confirmation that the 2 blood Phe results are > 600 µmol/L and all other eligibility criteria have been met, participants may be enrolled in the study on Day 1. During the Screening/Run-in and the Primary Treatment Phase (Part 1), participants will be required to maintain stable protein intake from both medical food and/or intact food.

The duration of Part 1 is 72 weeks, during which pegvaliase treatment will be initiated for participants in the active arm using an Induction/Titration/Maintenance (I/T/M) dosing regimen. The I/T/M dosing regimen consists of Induction/Titration Dosing (9 weeks) and

Maintenance Dosing. Required hospital setting visits during Part 1 will occur for all dosing visits for the first 8 weeks, every 4 weeks thereafter, and when the pegvaliase dose is escalated to 20, 40, or 60 mg/day. The assessments performed at the clinic visits include measurement of blood Phe, neurocognitive assessments, global impression items, PK blood draws (active participants only), and safety assessments.

Beginning Week 73, participants in the active treatment arm will continue to receive pegvaliase in the Extension Phase (Part 2) for up to 142 additional weeks (through Week 215). Beginning Week 73, participants in the control treatment arm will initiate pegvaliase treatment and, from Week 73 through Week 215, will follow the same schedule for dosing, study visits, and assessments that the active arm participants followed.

Brief Summary:

The purpose of Study 165-306 is to evaluate the safety and efficacy of pegvaliase and characterize the PK of pegvaliase in adolescent participants with PKU. Study details include:

Condition/Disease: PKU is a rare autosomal recessive genetic disorder caused by mutations in the phenylalanine hydroxylase (PAH) gene, leading to an absence or deficiency in PAH enzyme activity and subsequent elevation of the amino acid phenylalanine (Phe) in the blood, known as hyperphenylalaninemia (HPA) (Vockley 2014; Mitchell 2011). Deficiency of PAH results in an abnormally elevated concentration of Phe, which is toxic to the brain. High Phe levels during infancy and early childhood cause profound neurocognitive and developmental defects, and poorly controlled blood Phe levels in older children and adults are associated with learning disabilities, attention deficit hyperactivity disorder, behavioral problems, and psychiatric symptoms.

Study Hypothesis: The study hypothesis is that participants receiving pegvaliase will show a greater reduction in blood Phe following 72 weeks on study compared to participants in the diet-only control group.

Study Duration: The study duration is up to 222 weeks for participants randomized to active (pegvaliase) treatment. This includes a 4-week Screening/Run-in period, up to 214 weeks of pegvaliase treatment in Parts 1 and 2, and the Study Completion/Early Termination (SC/ET) visit 4 weeks after the final dose of pegvaliase. The study duration is also 222 weeks for participants randomized to control (diet-only) treatment. This includes a 4-week Screening/Run-in period, 72 weeks managing PKU with diet alone while following the Part 1 assessment schedule, 142 weeks of pegvaliase treatment following the same assessment schedule that participants in the active treatment arm followed, and the SC/ET visit 4 weeks after the final dose of pegvaliase.

Treatment Duration: The pegvaliase treatment duration will be 214 weeks for participants randomized to the active (pegvaliase) treatment arm (n=36). The pegvaliase treatment duration will be 142 weeks for participants randomized to the control (diet-only) treatment arm (n=18).

Health Measurement/Observation: Reduction in blood Phe.

Visit Frequency: For both active and control participants, hospital setting visits in Part 1 will occur for all dosing visits for the first 8 weeks, then every 4 weeks through Week 73 and for all dose escalations. For participants randomized to active (pegvaliase) treatment, clinic visits in Part 2 will occur every 4 weeks through Week 215 and for all dose escalations. For participants randomized to control (diet-only) treatment, hospital setting visits in Part 2 will occur for all dosing visits for the first 8 weeks, then every 4 weeks through Week 215. For participants randomized to active (pegvaliase) treatment in Part 1 and participants randomized to control (diet-only) treatment in Part 2, hospital setting visits will also occur when their dose level is escalated to 20, 40, and 60 mg/day.

Number of Participants:

Approximately 54 participants will be enrolled and randomized to the pegvaliase or diet-only groups in a 2:1 ratio. Assuming the mean (SD) reduction in Phe following 72 weeks on study is 640 (570) µmol/L in the pegvaliase group and 100 (250) µmol/L in the diet-only group, a total sample size of 54 participants will provide more than 90% power to detect a treatment difference, based on the two-sample T-test with unequal variance and a significance level of 0.05 (two sided). The assumption of treatment effect is based on clinical data from previous pegvaliase studies and a PKU registry study. See the Statistical Analysis Plan (SAP) for additional details on the sample size calculation.

Approximately 72 participants will be screened for study participation such that approximately 54 participants will be enrolled in the study.

Note: Potential participants who are screened for the purpose of determining eligibility for the study, but do not participate in the study, are not considered enrolled.

Treatment Groups and Duration:

The duration of the Primary Treatment Phase (Part 1) is 72 weeks, during which pegvaliase treatment will be initiated for participants randomized to the active (pegvaliase) treatment arm using an I/T/M dosing regimen. Induction will consist of a 4-week period when participants randomized to active drug will receive pegvaliase subcutaneously with a pre-filled syringe (PFS) at a fixed dose of 2.5 mg/week and during which the dosing regimen

will not be modified in response to blood Phe concentration. Once participants complete induction they will titrate up to a dose of 10 mg/day (investigators may, at their discretion, include dosing at a 5 mg/day dose level). The dosing frequency will be increased gradually during titration dosing to daily (7 days/week). During Maintenance Dosing the dose will be increased to 20 mg/day, 40 mg/day, and 60 mg/day depending upon individual blood Phe lowering efficacy (per the investigator's discretion). The dose should be increased to 40 mg/day if blood Phe is > 360 µmol/L (recommended) after 24 weeks dosing at 20 mg/day and increased to 60 mg/day if blood Phe is > 360 µmol/L (recommended) after 16 weeks dosing at 40 mg/day. If a participant's blood Phe is > 600 µmol/L after 16 weeks of continuous treatment with pegvaliase at 60 mg/day, pegvaliase should be permanently discontinued. BioMarin recommends that the target blood Phe levels should be based on individual needs per investigator's discretion, with a target blood Phe ≤ 600 µmol/L or lower. During the Primary Treatment Phase (Part 1), participants randomized to the control (diet-only) treatment arm will have their PKU managed by diet alone.

Following completion of Part 1, participants randomized to active (pegvaliase) treatment will enter the Extension Phase (Part 2), during which participants will continue dosing with pegvaliase at their optimal maintenance dose level for up to an additional 142 weeks (through Week 215) in order to evaluate the long-term safety and efficacy of pegvaliase in adolescent participants with PKU. Following completion of Part 1 (Week 72), participants randomized to control (diet-only) treatment will initiate pegvaliase treatment and, from Week 73 through Week 215, will follow the same schedule for dosing, study visits, and assessments that the active arm participants followed.

Data Monitoring Committee:

An independent Data Monitoring Committee (DMC) will monitor the safety of study participants. The DMC is an independent committee that acts in an advisory capacity to BioMarin.

1.2 Study Schema

Figure 1.2.1: Active (Pegvaliase) vs Control (Diet-Only) Treatment Arms (Randomized Treatment Assignment)

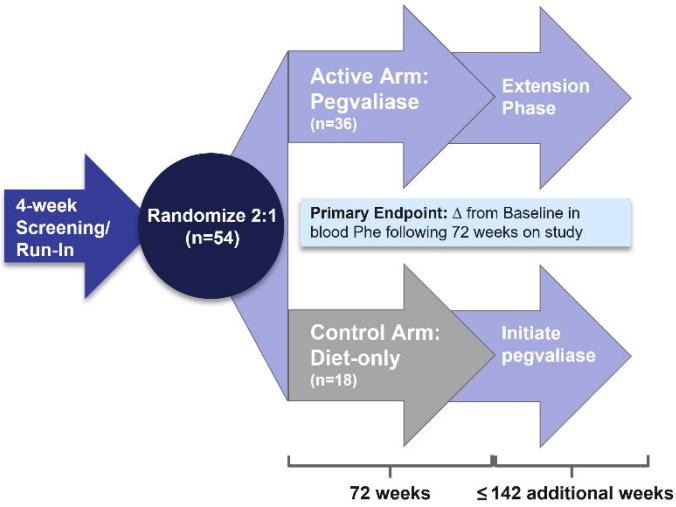
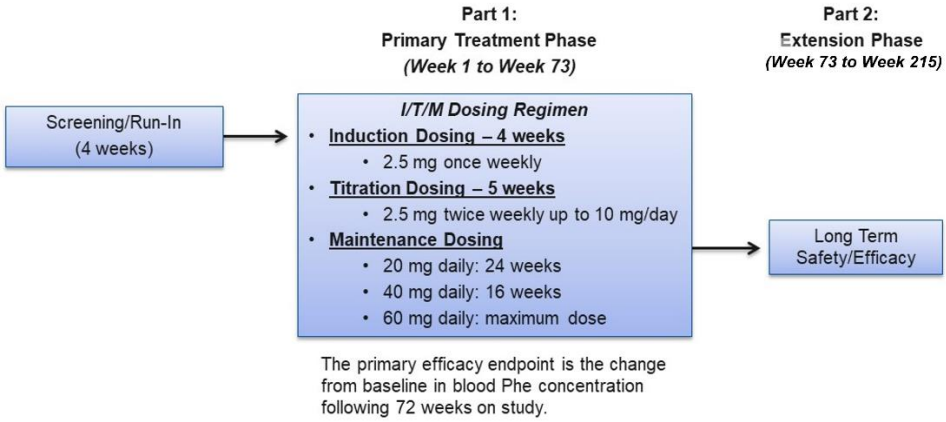


Figure 1.2.2: Dosing Schematic for Active (Pegvaliase) Treatment Arm



Note: The 18 control (diet only) participants will follow the Part 1 assessment schedule (except for pegvaliase dosing and PK/immunogenicity assessments) from Weeks 1 through Week 73 and then will initiate pegvaliase treatment and, from Week 73 through Week 215, will follow the same schedule for dosing, study visits, and assessments as the active arm participants.

Prior to dose escalation, the minimum amount of time pegvaliase can be dosed at 20 mg/day before escalating is 24 weeks and the minimum amount of time at 40 mg/day before escalating is 16 weeks. The dose should be increased to 40 mg/day if blood Phe is > 360 µmol/L (recommended) after 24 weeks dosing at 20 mg/day, and increased to 60 mg/day if blood Phe is > 360 µmol/L (recommended) after 16 weeks dosing at 40 mg/day. If a participant's blood Phe is > 600 µmol/L after 16 weeks of continuous treatment with pegvaliase at 60 mg/day, pegvaliase should be permanently discontinued.

1.3 Schedule of Activities (SoA)

Table 1.3.1: Schedule of Activities for Part 1 (Participants Randomized to Pegvaliase Treatment Arm)

	Study Week	Screen[b]	1	2	3	4	5	6	7	8	9	11	13	15	17	19	21	25	29	33	37	41	45	49	53	57	61	65	69	73	HRV[c]	SC/ET[d]
	Study Day	-28/-1	1	8	15	22	29	36	43	50	57	71	85	99	113	127	141	169	197	225	253	281	309	337	365	393	421	449	477	505		
Assessment [a]	Window (Days)			±3	±3	±3	±3	±3	±3	±3	±3	±3	±7	±3	±7	±3	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7		
Informed consent		X																														
Drug administration training [e]			X																													
Study drug observer training [f]		X																														
Demographics		X																														
Medical history [g]		X																														
HIV, hepatitis B and C screens [h]		X																														
PAH genotyping [i]			X																													
12 lead ECG		X	X																													X
Physical examination [j]		X	X	X	X	X	X	X	X	X	X		X		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Weight		X	X	X	X	X	X	X	X	X	X		X		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Height		X	X				X				X		X		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
Vital signs		X	X	X	X	X	X	X	X	X	X		X		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Clinical safety laboratory tests [k]		X	X	X	X	X	X	X	X	X	X		X		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
C-reactive protein		X	X								X				X			X		X		X		X		X		X		X	X	X
Erythrocyte sedimentation rate [l]		X	X																													X
Serum cortisol [m]			X															X						X						X		X
Complements C3 and C4			X				X				X		X		X		X	X		X		X		X		X		X		X	X	X
Tryptase			X																												X	
Urine albumin/creatinine ratio [n]			X								X				X			X		X		X		X		X		X		X	X	X
Urine N-methylhistamine			X																												X	

Assessment [a]	Study Week	Screen[b]	1	2	3	4	5	6	7	8	9	11	13	15	17	19	21	25	29	33	37	41	45	49	53	57	61	65	69	73	HRV [c]	SC/ET [d]
	Study Day	-28/-1	1	8	15	22	29	36	43	50	57	71	85	99	113	127	141	169	197	225	253	281	309	337	365	393	421	449	477	505		
	Window (Days)			±3	±3	±3	±3	±3	±3	±3	±3	±3	±7	±3	±7	±3	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7		
Urine pregnancy test [o]		X	X	X	X	X	X	X	X	X	X		X		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
3-day diet diary [p]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
PK (plasma pegvaliase) [q]			X				X				X		X		X		X	X		X		X		X		X		X			X
Blood for exploratory analyses [i]			X				X				X		X		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
Blood Phe, tyrosine [r]		X [b]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Immunogenicity assessments [s]			X	X	X	X	X	X	X	X	X		X		X		X	X		X		X		X		X		X		X	X
ADHD RS-IV [t]			X										X					X			X			X			X			X		X
BRIEF2 [t]			X										X					X			X			X			X			X		X
PKU-SSIS [u,z]			X										X					X			X			X			X			X		X
PGIS [u,z]			X										X					X			X			X			X			X		X
PGIC [u]																					X									X		X
CaGIS [t,z]			X										X					X			X			X			X			X		X
CaGIC [t]																					X									X		X
CGIS [v,z]			X										X					X			X			X			X			X		X
Weekly Phone Call (non-visit weeks) [w]																																
Adverse events [x]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant medication [x]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Administer study drug [y]			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		

ADHD RS-IV, attention deficit hyperactivity disorder rating scale IV; BRIEF2, Behavior Rating Inventory of Executive Function, Second Edition; EC/IRB, Ethics Committee/Institutional Review Board; ECG, electrocardiogram; eCRF, electronic Case Report Form; HRV, hypersensitivity reaction visit; PAH, phenylalanine hydroxylase; PE, physical examination; PK, pharmacokinetics; PKU, phenylketonuria; SC/ET, Study Completion/Early Termination visit.

[a] Scheduled visits take place at the hospital setting (for all dosing visits for the first 8 weeks, and for all dose escalation visits), clinic, or, with the approval of the investigator or designee, virtually. For virtual visits the investigator/designee performs assessments while online with the participant, apart from specimen collections and vital signs, which are performed by home healthcare personnel. Non-clinic visits are via telephone. Assessments are performed pre-dose unless otherwise specified.

[b] After written informed consent, screening assessments should be performed within 28 days prior to Part 1, Day 1. Participants will be assessed for blood Phe concentration during Screening/Run-in with 2 measurements 7 to 10 days apart. These 2 blood Phe measurements should be scheduled to occur: (1) in the Day -25 to Day -23 window; and (2) 7 to 10 days later (ie, in the Day -18 to Day -13 window).

[c] If the investigator determines that the NCI-CTCAE Grade ≥ 3 hypersensitivity reaction is related to administration with study drug, the participant will be asked to return to the clinic within 24 hours of event onset for a hypersensitivity reaction visit (HRV). Sampling for anti-pegvaliase IgE should be performed 8 to 24 hours after event onset and before the next dose of study drug.

[d] Participants should perform the Study Completion (SC) Visit 4 weeks after the last dose of study drug or after completion of study participation, whichever occurs last. For participants who terminate from the study early, the Early Termination (ET) Visit occurs within 4 weeks of the last study drug dose or after withdrawal from study participation, whichever occurs last. Participants who discontinue from study drug early will be asked to continue to complete the remaining study assessments until study completion as long as such continued participation will not detrimentally affect the health, safety, and welfare of the participant per investigator determination.

[e] Once participant/caregiver competence is documented, the participant/caregiver administers study drug daily.

[f] Must be completed prior to administration of the first dose of study drug. Additional observers identified after screening must be trained.

[g] PKU history includes highest blood Phe (and age).

[h] Participants with documented negative results within the last 4 weeks do not need to be retested.

[i] Sample collection should be performed where allowed by country-specific regulations and EC/IRB.

[j] A full physical examination (PE) will be performed at Screening, Baseline, Week 73, and the SC/ET visit. A brief PE will be performed at other scheduled visits. A full PE will include, at a minimum, general appearance (head, eyes, ears, nose, and throat) and an assessment of cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, musculoskeletal, and neurologic systems. A brief PE may be performed at the discretion of the investigator based on the participant's clinical condition and may include, but is not limited to, general appearance and an assessment of cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, musculoskeletal, and neurologic systems.

[k] It is recommended that urine samples are obtained as a first or second morning void. In the event of elevated urinary protein on a test result, a repeat urinalysis should be performed. This repeat urine sample must be performed in the morning at the first or second morning void to allow for accurate test results and may be performed by a home healthcare nurse.

[l] To be done by a local laboratory.

[m] Serum cortisol samples should be taken before study drug administration in the morning before 10:00 am. A home healthcare nurse may collect samples for participant convenience. If 2 results are low and abnormal, the participant will be asked to perform a low-dose conventional ACTH stimulation test or the ACTH test method based on the clinical practice at the site.

[n] It is recommended that urine samples be obtained as a first or second morning void. Participants with a confirmed urine/albumin creatinine ratio of ≥ 100 mg/g should be referred to a nephrologist for consultation if results were within normal range at Baseline. Participants who had elevated results at Baseline followed by a confirmed subsequent increase of 100-200 mg/g from Baseline should also be referred to a nephrologist for consultation.

[o] To be done locally (if applicable). If urine pregnancy test is positive or equivocal, serum pregnancy test (central laboratory) must be done.

[p] Participants should record at home all food, beverages, special low-protein foods, and medical foods consumed during the 3 consecutive days preceding a study visit. No change in protein (dietary and medical food) intake is allowed in Part 1. During Screening/Run-in, 3-day diet diary assessments are performed 3 times, each time covering the 3-day periods prior to blood Phe assessments (and collected on the day of those assessments). The 3 Screening/Run-in blood Phe assessments are scheduled to occur (1) at Day -25 to Day -23; (2) 7 to 10 days later (ie, in the Day -18 to Day -13 window); and (3) at Day 1 pre-dose. Thus, the 3 day diet assessments will assess diet for (1) the 3 days within the Day -28 to Day -23 window immediately prior to the first blood Phe assessment; (2) the 3 days within the Day -21 to Day -15 window immediately prior to the second blood Phe assessment; and (3) for Day -3 to Day 1.

[q] Blood is collected before study drug administration (pre-dose) within 2 hours of dosing. Intensive PK sampling will be performed at Week 73 and at dose escalation visits in all participants. Samples will be taken at pre-dose (within 2 hours of dosing); at 2, 4, 8, and 12 hours (± 30 minutes) post-dose; and 24 hours (± 2 hours) post dose. The 24-hour sample will be taken prior to the next daily dose.

[r] Blood is collected for blood Phe analysis after fasting a minimum of 2.5 hours. In addition to the blood draws for blood Phe analysis collected in the clinic and shown in the SoA, there are 5 required blood collections that may be drawn either at the clinic or elsewhere (eg, at home). These are to be collected at Weeks 11, 15, and 19, and 2 weeks prior to the planned escalations to 40 and 60 mg/day. Three (3) Day diet diary data collection must be done prior to all Phe collections.

[s] Immunogenicity assays include total anti-pegvaliase antibodies (TAb), anti-PAL IgG, anti-PAL IgM, anti-PEG IgM, anti-PEG IgG, and neutralizing antibodies (NAb). At the HRV, only anti-pegvaliase IgE will be assessed.

[t] Completed by parent/guardian. Assessments should be completed before other study visit assessments (eg, vital signs, blood draw for blood Phe, and study drug administration).

[u] Completed by participant. Assessments should be completed before other study visit assessments (eg, vital signs, blood draw for blood Phe, and study drug administration).

[v] Completed by the investigator or designee clinician. Assessments should be completed before other study visit assessments (eg, vital signs, blood draw for blood Phe, and study drug administration). Visits where CGIS will be assessed should take place in the hospital setting or clinic.

[w] Between scheduled visits, the clinic staff will contact the participant via phone weekly to monitor if the participant is experiencing problems with drug administration, to ask about any AEs or concomitant medications, and to answer questions.

[x] AEs and concomitant medications should be noted whenever a participant is assessed by study personnel. Following signed informed consent and prior to the first dose of pegvaliase, only SAEs associated with study procedures are collected. After the first dose all AEs and SAEs are collected until 4 weeks after the last study drug dose

or the Early Termination Visit, whichever occurs last. If there is a severe (Grade 3) injection site reaction, the participant may be referred to a dermatologist for consultation and a skin biopsy (optional; if clinically indicated). If photographs of the severe injection site reaction are taken, they may be collected by the sponsor.

[y] Participants must be premedicated with an H1 antagonist, and an H2 antagonist, and an antipyretic approximately 2 to 3 hours prior to each dose of study drug until they reach the Maintenance Phase, defined as when participants achieve blood Phe ≤ 600 µmol/L while they are on a stable dose of pegvaliase. Premedication may be considered during the Maintenance Phase at the investigator's discretion, eg, approximately 2 to 3 hours prior to study drug for 1 week upon reintroduction of study drug, upon resolution of an AE, following any dose interruption of ≥ 4 days, and for any dose increases in the Extension Phase. Participants may also be premedicated at any other time in the study per investigator discretion. When an NSAID is administered as a premedication, it should be given with food. For non-clinic visits, participants are asked about study drug administration.

[z] Initial assessments of the PKU-SSIS, PGIS, CaGIS, and CGIS will be at the first visit following implementation/adoption of Protocol Amendment 2.

Table 1.3.2: Schedule of Activities for Part 2 (Extension Phase) Weeks 73-149 (Participants Randomized to Pegvaliase Treatment Arm)

Assessment [a]	Study Week	73[b]	77	81	85	89	93	97	101	105	109	113	117	121	125	129	133	137	141	145	149	HRV[c]	SC/ET[d]
	Study Day	505	533	561	589	617	645	673	701	729	757	785	813	841	869	897	925	953	981	1009	1037		
	Window (Days)	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7		
Study drug observer training [e]																							
12 lead ECG																							X
Physical examination [f]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Weight	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Height	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			X
Vital signs	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Clinical safety laboratory tests [g]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
C-reactive protein	X		X		X		X		X		X		X		X		X		X		X	X	X
Erythrocyte sedimentation rate [h]																							X
Serum cortisol [i]	X						X						X										X
Complements C3 and C4	X		X		X		X		X		X		X		X		X		X		X	X	X
Tryptase																						X	
Urine albumin/creatinine ratio [j]	X		X		X		X		X		X		X		X		X		X		X	X	X
Urine N-methylhistamine																						X	
Urine pregnancy test [k]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
3-day diet diary [l]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			X
PK (plasma pegvaliase) [m]	X		X		X		X		X		X		X		X		X		X				X
Blood for exploratory analyses [n]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			X
Blood Phe, tyrosine [o]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Immunogenicity assessments [p]	X		X		X		X		X		X		X		X		X		X		X	X	X
ADHD RS-IV [q]	X			X			X			X			X			X			X				X
BRIEF2 [q]	X			X			X			X			X			X			X				X
PKU-SSIS [r]	X			X			X			X			X			X			X				X

Assessment [a]	Study Week	73[b]	77	81	85	89	93	97	101	105	109	113	117	121	125	129	133	137	141	145	149	HRV[c]	SC/ET[d]
	Study Day	505	533	561	589	617	645	673	701	729	757	785	813	841	869	897	925	953	981	1009	1037		
	Window (Days)	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7		
PGIS [r]		X			X			X			X			X			X			X			X
PGIC [r]		X									X									X			X
CaGIS [q]		X			X			X			X			X			X			X			X
CaGIC [q]		X									X									X			X
CGIS [s]		X			X			X			X			X			X			X			X
Weekly Phone Call (non-visit weeks) [t]																							
Adverse events [u]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant medication [u]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Administer study drug [v]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		

ADHD RS-IV, attention deficit hyperactivity disorder rating scale IV; BRIEF2, Behavior Rating Inventory of Executive Function, Second Edition; ECG, electrocardiogram; eCRF, electronic Case Report Form; HRV, hypersensitivity reaction visit; PE, physical examination; PK, pharmacokinetics; PKU, phenylketonuria; SC/ET, Study Completion/Early Termination visit.

[a] Scheduled visits take place at the hospital setting (for all dose escalation visits and visits where the CGIS assessments will be performed), clinic, or, with the approval of the investigator or designee, virtually. For virtual visits the investigator/designee performs assessments while online with the participant, apart from specimen collections and vital signs, which are performed by home healthcare personnel. Non-clinic visits are via telephone. Assessments are performed pre-dose unless otherwise specified.

[b] The Week 73 visit marks both the end of the Primary Treatment Phase (Part 1) and the beginning of the Extension Phase (Part 2). The pre-dose assessments constituting the end of the Primary Treatment Phase and the administration of study drug constituting the beginning of the Extension Phase are shown in the Week 73 columns of both Table 1.3.1 and Table 1.3.2.

[c] If the investigator determines that the NCI-CTCAE Grade ≥ 3 hypersensitivity reaction is related to administration with study drug, the participant will be asked to return to the clinic within 24 hours of event onset for a hypersensitivity reaction visit (HRV). Sampling for anti-pegvaliase IgE should be performed 8 to 24 hours after event onset and before the next dose of study drug.

[d] Participants should perform the Study Completion (SC) Visit 4 weeks after the last dose of study drug or after completion of study participation, whichever occurs last. For participants who terminate from the study early, the Early Termination (ET) Visit occurs within 4 weeks of the last study drug dose or after withdrawal from study participation, whichever occurs last. Participants who discontinue from study drug early will be asked to continue to complete the remaining study assessments until study completion as long as such continued participation will not detrimentally affect the health, safety, and welfare of the participant per investigator determination.

[e] Additional observers identified after screening must be trained.

[f] A full PE will be performed at Week 73, Week 145, and the SC/ET visit. A brief PE will be performed at other visits. A full PE will include, at a minimum, general appearance (head, eyes, ears, nose, and throat) and an assessment of cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, musculoskeletal, and neurologic systems. A brief PE may be performed at the discretion of the investigator based on the participant's clinical condition and may include, but is not limited to, general appearance and an assessment of cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, musculoskeletal, and neurologic systems.

[g] It is recommended that urine samples are obtained as a first or second morning void. In the event of elevated urinary protein on a test result, a repeat urinalysis should be performed. This repeat urine sample must be performed in the morning at the first or second morning void to allow for accurate test results and may be performed by a home healthcare nurse.

[h] To be done by a local laboratory.

[i] Serum cortisol samples should be taken before study drug administration in the morning before 10:00 am. A home healthcare nurse may collect samples for participant convenience. If 2 results are low and abnormal, the participant will be asked to perform additional sampling for serum cortisol and a low-dose conventional ACTH stimulation test or the ACTH test method based on the clinical practice at the site.

[j] It is recommended that urine samples be obtained as a first or second morning void. Participants with a confirmed urine/albumin creatinine ratio of ≥ 100 mg/g should be referred to a nephrologist for consultation if results were within normal range at Baseline. Participants who had elevated results at Baseline followed by a confirmed subsequent increase of 100-200 mg/g from Baseline should also be referred to a nephrologist for consultation.

[k] To be done locally (if applicable). If urine pregnancy test is positive or equivocal, serum pregnancy test (central laboratory) must be done.

[l] Participants should record at home all food, beverages, special low-protein foods, and medical foods consumed during the 3 consecutive days preceding a study visit.

[m] Blood is collected before study drug administration (pre-dose) within 2 hours of dosing. Intensive PK sampling will be performed at Week 73 and at dose escalation visits in all participants. Samples will be taken at pre-dose (within 2 hours of dosing); at 2, 4, 8, and 12 hours (± 30 minutes) post-dose; and 24 hours (± 2 hours) post dose. The 24-hour sample will be taken prior to the next daily dose.

[n] Sample collection should be performed where allowed by country-specific regulations and EC/IRB.

[o] Blood is collected for blood Phe analysis after fasting a minimum of 2.5 hours.

[p] Immunogenicity assays include total anti-pegvaliase antibodies (TAb), anti-PAL IgG, anti-PAL IgM, anti-PEG IgM, anti-PEG IgG, and neutralizing antibodies (NAb). At the HRV, only anti-pegvaliase IgE will be assessed.

[q] Completed by parent/guardian. Assessments should be completed before other study visit assessments (eg, vital signs, blood draw for blood Phe, and study drug administration).

[r] Completed by participant. Assessments should be completed before other study visit assessments (eg, vital signs, blood draw for blood Phe, and study drug administration).

[s] Completed by the investigator or designee clinician. Assessments should be completed before other study visit assessments (eg, vital signs, blood draw for blood Phe, and study drug administration). Visits where CGIS will be assessed should take place in the hospital setting or clinic.

[t] Between scheduled clinic visits, the clinic staff will contact the participant via phone weekly to monitor if the participant is experiencing problems with drug administration, to ask about any AEs or concomitant medications, and to answer questions.

[u] AEs and concomitant medications should be noted whenever a participant is assessed by study personnel. All AEs and SAEs are collected until 4 weeks after the last study drug dose or the Study Completion Visit/Early Termination visit, whichever occurs last. If there is a severe (Grade 3) injection site reaction, the participant may be referred to a dermatologist for consultation and a skin biopsy (optional; if clinically indicated). If photographs of the severe injection site reaction are taken, they may be collected by the sponsor.

[v] Participants must be premedicated with an H1 antagonist, an H2 antagonist, and an antipyretic approximately 2 to 3 hours prior to each dose of study drug until they reach the Maintenance Phase, defined as when participants achieve blood Phe $\leq$ 600 µmol/L while they are on a stable dose of pegvaliase. Premedication may be considered during the Maintenance Phase at the investigator's discretion, eg, approximately 2 to 3 hours prior to study drug for 1 week upon reintroduction of study drug, upon resolution of an AE, following any dose interruption of $\geq$ 4 days, and for any dose increases in the Extension Phase. Participants may also be premedicated at any other time in the study per investigator discretion. When an NSAID is administered as a premedication, it should be given with food. For non-clinic visits, participants are asked about study drug administration.

Table 1.3.3: Schedule of Activities for Part 2 (Extension Phase) Weeks 153-215 (Participants Randomized to Pegvaliase Treatment Arm)

Assessment [a]	Study Week	153	157	161	165	169	173	177	181	185	189	193	197	201	205	209	213	215	HRV[b]	SC/ET[c]
	Study Day	1065	1093	1121	1149	1177	1205	1233	1261	1289	1317	1345	1373	1401	1429	1457	1485	1499		
	Window (Days)	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7		
Study drug observer training [d]																				
12 lead ECG																				X
Physical examination [e]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Weight		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Height		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
Vital signs		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Clinical safety laboratory tests [f]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
C-reactive protein		X		X		X		X		X		X		X		X		X	X	X
Erythrocyte sedimentation rate [g]																				X
Serum cortisol [h]		X									X									X
Complements C3 and C4		X		X		X		X		X	X	X		X		X		X	X	X
Tryptase																			X	
Urine albumin/creatinine ratio [i]		X		X		X		X		X		X		X		X		X	X	X
Urine N-methylhistamine																			X	
Urine pregnancy test [j]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
3-day diet diary [k]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
PK (plasma pegvaliase) [l]		X		X		X		X		X		X		X		X		X		X
Blood for exploratory analyses [m]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
Blood Phe, tyrosine [n]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Immunogenicity assessments [o]		X		X		X		X		X		X		X		X		X	X	X
ADHD RS-IV [p]			X			X			X			X			X			X		X
BRIEF2 [p]			X			X			X			X			X			X		X
PKU-SSIS [q]			X			X			X			X			X			X		X

Assessment [a]	Study Week	153	157	161	165	169	173	177	181	185	189	193	197	201	205	209	213	215	HRV[b]	SC/ET[c]
	Study Day	1065	1093	1121	1149	1177	1205	1233	1261	1289	1317	1345	1373	1401	1429	1457	1485	1499		
	Window (Days)	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7		
PGIS [q]			X			X			X			X			X			X		X
PGIC [q]									X									X		X
CaGIS [p]			X			X			X			X			X			X		X
CaGIC [p]									X									X		X
CGIS [r]			X			X			X			X			X			X		X
Weekly Phone Call (non-visit weeks) [s]																				
Adverse events [t]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant medication [t]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Administer study drug [u]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		

ADHD RS-IV, attention deficit hyperactivity disorder rating scale IV; BRIEF2, Behavior Rating Inventory of Executive Function, Second Edition; ECG, electrocardiogram; eCRF, electronic Case Report Form; HRV, hypersensitivity reaction visit; PE, physical examination; PK, pharmacokinetics; PKU, phenylketonuria; NSAID, non-steroidal anti-inflammatory drug; SC/ET, Study Completion/Early Termination visit.

[a] Scheduled visits take place at the hospital setting (for all dose escalation visits and visits where the CGIS assessments will be performed), clinic, or, with the approval of the investigator or designee, virtually. For virtual visits the investigator/designee performs assessments while online with the participant, apart from specimen collections and vital signs, which are performed by home healthcare personnel. Non-clinic visits are via telephone. Assessments are performed pre-dose unless otherwise specified.

[b] If the investigator determines that the NCI-CTCAE Grade ≥ 3 hypersensitivity reaction is related to administration with study drug, the participant will be asked to return to the clinic within 24 hours of event onset for a hypersensitivity reaction visit (HRV). Sampling for anti-pegvaliase IgE should be performed 8 to 24 hours after event onset and before the next dose of study drug.

[c] Participants should perform the Study Completion (SC) Visit 4 weeks after the last dose of study drug or after completion of study participation, whichever occurs last. For participants who terminate from the study early, the Early Termination (ET) Visit occurs within 4 weeks of the last study drug dose or after withdrawal from study participation, whichever occurs last. Participants who discontinue from study drug early will be asked to continue to complete the remaining study assessments until study completion as long as such continued participation will not detrimentally affect the health, safety, and welfare of the participant per investigator determination.

[d] Additional observers identified after screening must be trained.

[e] A full PE will be performed at the SC/ET visit. A brief PE will be performed at other visits. A full PE will include, at a minimum, general appearance (head, eyes, ears, nose, and throat) and an assessment of cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, musculoskeletal, and neurologic systems. A brief PE may

be performed at the discretion of the investigator based on the participant's clinical condition and may include, but is not limited to, general appearance and an assessment of cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, musculoskeletal, and neurologic systems.

[f] It is recommended that urine samples are obtained as a first or second morning void. In the event of elevated urinary protein on a test result, a repeat urinalysis should be performed. This repeat urine sample must be performed in the morning at the first or second morning void to allow for accurate test results and may be performed by a home healthcare nurse.

[g] To be done by a local laboratory.

[h] Serum cortisol samples should be taken before study drug administration in the morning before 10:00 am. A home healthcare nurse may collect samples for participant convenience. If 2 results are low and abnormal, the participant will be asked to perform additional sampling for serum cortisol and a low-dose conventional ACTH stimulation test or the ACTH test method based on the clinical practice at the site.

[i] It is recommended that urine samples be obtained as a first or second morning void. Participants with a confirmed urine/albumin creatinine ratio of ≥ 100 mg/g should be referred to a nephrologist for consultation if results were within normal range at Baseline. Participants who had elevated results at Baseline followed by a confirmed subsequent increase of 100-200 mg/g from Baseline should also be referred to a nephrologist for consultation.

[j] To be done locally (if applicable). If urine pregnancy test is positive or equivocal, serum pregnancy test (central laboratory) must be done.

[k] Participants should record at home all food, beverages, special low-protein foods, and medical foods consumed during the 3 consecutive days preceding a study visit. No change in protein (dietary and medical food) intake is allowed in Part 1.

[l] Blood is collected before study drug administration (pre-dose) within 2 hours of dosing. Intensive PK sampling will be performed at dose escalation visits in all participants. Samples will be taken at pre-dose (within 2 hours of dosing); at 2, 4, 8, and 12 hours (± 30 minutes) post-dose; and 24 hours (± 2 hours) post dose. The 24-hour sample will be taken prior to the next daily dose.

[m] Sample collection should be performed where allowed by country-specific regulations and EC/IRB.

[n] Blood is collected for blood Phe analysis after fasting a minimum of 2.5 hours.

[o] Immunogenicity assays include total anti-pegvaliase antibodies (TAb), anti-PAL IgG, anti-PAL IgM, anti-PEG IgM, anti-PEG IgG, and neutralizing antibodies (NAb). At the HRV, only anti-pegvaliase IgE will be assessed.

[p] Completed by parent/guardian. Assessments should be completed before other study visit assessments (eg, vital signs, blood draw for blood Phe, and study drug administration).

[q] Completed by participant. Assessments should be completed before other study visit assessments (eg, vital signs, blood draw for blood Phe, and study drug administration).

[r] Completed by the investigator or designee clinician. Assessments should be completed before other study visit assessments (eg, vital signs, blood draw for blood Phe, and study drug administration). Visits where CGIS will be assessed should take place in the hospital setting or clinic.

[s] Between scheduled clinic visits, the clinic staff will contact the participant weekly to monitor if the participant is experiencing problems with drug administration, to ask about any AEs or concomitant medications, and to answer questions.

[t] AEs and concomitant medications should be noted whenever a participant is assessed by study personnel. All AEs and SAEs are collected until 4 weeks after the last study drug dose or the Study Completion Visit/Early Termination visit, whichever occurs last. If there is a severe (Grade 3) injection site reaction, the participant may be referred to a dermatologist for consultation and a skin biopsy (optional; if clinically indicated). If photographs of the severe injection site reaction are taken, they may be collected by the sponsor.

[u] Participants must be premedicated with an H1 antagonist, and an H2 antagonist, and an antipyretic approximately 2 to 3 hours prior to each dose of study drug until they reach the Maintenance Phase, defined as when participants achieve blood Phe ≤ 600 µmol/L while they are on a stable dose of pegvaliase. Premedication may be considered during the Maintenance Phase at the investigator's discretion, eg, approximately 2 to 3 hours prior to study drug for 1 week upon reintroduction of study drug, upon resolution of an AE, following any dose interruption of ≥ 4 days, and for any dose increases in the Extension Phase. Participants may also be premedicated at any other time in the study per investigator discretion. When an NSAID is administered as a premedication, it should be given with food. For non-clinic visits, participants are asked about study drug administration.

Table 1.3.4: Schedule of Activities for Part 1 (Participants Randomized to Diet-Only Treatment Arm)

Assessment [a]	Study Week	Screen[b]	1	2	3	4	5	6	7	8	9	13	17	21	25	29	33	37	41	45	49	53	57	61	65	69	73	SC/ET[c]
	Study Day	-28/-1	1	8	15	22	29	36	43	50	57	85	113	141	169	197	225	253	281	309	337	365	393	421	449	477	505	
	Window (Days)			±3	±3	±3	±3	±3	±3	±3	±3	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±3	
Informed consent		X																										
Drug administration training [d]																											X	
Study drug observer training [e]																											X	
Demographics		X																										
Medical history (allergy, PKU) [f]		X																										
HIV, hepatitis B and C screens [g]		X																										
PAH genotyping [h]			X																									
12 lead ECG		X	X																								X	X
Physical examination [i]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Weight		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Height		X	X				X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital signs		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Clinical safety laboratory tests [j]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
C-reactive protein		X	X								X		X		X		X		X		X		X		X		X	X
Erythrocyte sedimentation rate [k]		X	X																								X	X
Serum cortisol [l]			X												X						X						X	X
Complements C3 and C4			X				X				X		X		X		X		X		X		X		X		X	X
Tryptase			X																								X	
Urine albumin/creatinine ratio [m]			X								X		X		X		X		X		X		X		X		X	X
Urine pregnancy test [n]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
3-day diet diary [o]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Blood for exploratory analyses [h]			X				X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Blood Phe, tyrosine [p]		X [b]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
ADHD RS-IV [q]			X									X			X			X			X			X			X	X

Assessment [a]	Study Week	Screen[b]	1	2	3	4	5	6	7	8	9	13	17	21	25	29	33	37	41	45	49	53	57	61	65	69	73	SC/ET[c]
	Study Day	-28/-1	1	8	15	22	29	36	43	50	57	85	113	141	169	197	225	253	281	309	337	365	393	421	449	477	505	
	Window (Days)			±3	±3	±3	±3	±3	±3	±3	±3	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±3	
BRIEF2 [q]			X									X			X			X			X			X			X	X
PKU-SSIS [r,v]			X									X			X			X			X			X			X	X
PGIS [r,v]			X									X			X			X			X			X			X	X
PGIC [r]																		X									X	X
CaGIS [q,v]			X									X			X			X			X			X			X	X
CaGIC [q]																		X									X	X
CGIS [s,v]			X									X			X			X			X			X			X	X
Weekly Phone Call (non-visit weeks) [t]																												
Adverse events [u]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant medication [u]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

ADHD RS-IV, attention deficit hyperactivity disorder rating scale IV; BRIEF2, Behavior Rating Inventory of Executive Function, Second Edition; EC/IRB, Ethics Committee/Institutional Review Board; ECG, electrocardiogram; eCRF, electronic Case Report Form; HRV, hypersensitivity reaction visit; PAH, phenylalanine hydroxylase; PE, physical examination; PK, pharmacokinetics; PKU, phenylketonuria; SC/ET, Study Completion/Early Termination visit.

[a] Scheduled visits take place at the clinic or, with the approval of the investigator or designee, virtually. For virtual visits the investigator/designee performs assessments while online with the participant, apart from specimen collections and vital signs, which are performed by home healthcare personnel. Non-clinic visits are via telephone.

[b] After written informed consent, screening assessments should be performed within 28 days prior to Part 1, Day 1. Participants will be assessed for blood Phe concentration during Screening/Run-in with 2 measurements 7 to 10 days apart. These 2 blood Phe measurements should be scheduled to occur: (1) in the Day -25 to Day -23 window; and (2) 7 to 10 days later (ie, in the Day -18 to Day -13 window).

[c] Participants should perform the Study Completion (SC) Visit 4 weeks after the last dose of study drug or after completion of study participation, whichever occurs last. For participants who terminate from the study early, the Early Termination (ET) Visit occurs within 4 weeks of the last study drug dose or after withdrawal from study participation, whichever occurs last. Participants who discontinue from study drug early will be asked to continue to complete the remaining study assessments until study completion as long as such continued participation will not detrimentally affect the health, safety, and welfare of the participant per investigator determination.

[d] Once participant/caregiver competence is documented, the participant/caregiver administers study drug.

[e] Must be completed prior to administration of the first dose of study drug in Part 2. Additional observers identified after Screening must be trained.

[f] PKU history includes highest blood Phe (and age).

[g] Participants with documented negative results within the last 4 weeks do not need to be retested.

[h] Sample collection should be performed where allowed by country-specific regulations and EC/IRB.

[i] A full PE will be performed at Screening, Baseline, Week 73, and the SC/ET visit. A brief PE will be performed at other visits. A full PE will include, at a minimum, general appearance (head, eyes, ears, nose, and throat), and an assessment of cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, musculoskeletal, and neurologic systems. A brief PE may be performed at the discretion of the investigator based on the participant's clinical condition and may include, but is not limited to, general appearance and an assessment of cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, musculoskeletal, and neurologic systems.

[j] It is recommended that urine samples are obtained as a first or second morning void. In the event of elevated urinary protein on a test result, a repeat urinalysis should be performed. This repeat urine sample must be performed in the morning at the first or second morning void to allow for accurate test results and may be performed by a home healthcare nurse.

[k] To be done by a local laboratory.

[l] Serum cortisol samples should be taken before study drug administration in the morning before 10:00 am. A home healthcare nurse may collect samples for participant convenience. If 2 results are low and abnormal, the participant will be asked to perform additional sampling for serum cortisol and a low-dose conventional ACTH stimulation test or the ACTH test method based on the clinical practice at the site.

[m] It is recommended that urine samples be obtained as a first or second morning void. Participants with a confirmed urine/albumin creatinine ratio of ≥ 100 mg/g should be referred to a nephrologist for consultation if results were within normal range at Baseline. Participants who had elevated results at Baseline followed by a confirmed subsequent increase of 100-200 mg/g from Baseline should also be referred to a nephrologist for consultation.

[n] To be done locally (if applicable). If urine pregnancy test is positive or equivocal, serum pregnancy test (central laboratory) must be done.

[o] Participants should record at home all food, beverages, special low-protein foods, and medical foods consumed during the 3 consecutive days preceding a study visit. No change in protein (dietary and medical food) intake is allowed in Part 1. During Screening/Run-in, 3-day diet diary assessments are performed 3 times, each time covering the 3-day periods prior to blood Phe assessments (and collected on the day of those assessments). The 3 Screening/Run-in blood Phe assessments are scheduled to occur (1) at Day -25 to Day -23; (2) 7 to 10 days later (ie, in the Day -18 to Day -13 window); and (3) at Day 1 pre-dose. Thus, the 3 day diet assessments will assess diet for (1) the 3 days within the Day -28 to Day -23 window immediately prior to the first blood Phe assessment; (2) the 3 days within the Day -21 to Day -15 window immediately prior to the second blood Phe assessment; and (3) for Day -3 to Day 1.

[p] Blood is collected for blood Phe analysis after fasting a minimum of 2.5 hours. In addition to the blood draws for blood Phe analysis collected in the clinic and shown in the SoA, there are 3 required blood collections that may be drawn either at the clinic or elsewhere (eg, at home). Diet diary data collection must be done prior to all Phe collections.

[q] Completed by parent/guardian. Assessments should be completed before other study visit assessments (eg, vital signs, blood draw for blood Phe, and study drug administration).

[r] Completed by participant. Assessments should be completed before other study visit assessments (eg, vital signs, blood draw for blood Phe, and study drug administration).

[s] Completed by the investigator or designee clinician. Assessments should be completed before other study visit assessments (eg, vital signs, blood draw for blood Phe, and study drug administration). Visits where CGIS will be assessed should take place in the hospital setting or clinic.

[t] Between scheduled clinic visits, the clinic staff will contact the participant weekly to ask about any AEs or concomitant medications, and to answer questions.

[u] AEs and concomitant medications should be noted whenever a participant is assessed by study personnel. Following signed informed consent all AEs and SAEs are collected throughout Part 1 or until the Early Termination Visit, whichever occurs last.

[v] Initial assessments of the PKU-SSIS, PGIS, CaGIS, and CGIS will be at the first visit following implementation/adoption of Protocol Amendment 2.

Table 1.3.5: Schedule of Activities for Part 2 (Primary Treatment Phase – Weeks 73-145)
(Pegvaliase Treatment for Participants Randomized to Diet-Only Treatment Arm)

Assessment [a]	Study Week	73[b]	74	75	76	77	78	79	80	81	83	85	87	89	91	93	97	101	105	109	113	117	121	125	129	133	137	141	145	HRV[c]	SC/ET[d]
	Study Day	505	512	519	526	533	540	547	554	561	575	589	603	617	631	645	673	701	729	757	785	813	841	869	897	925	953	981	1009		
	Window (Days)	±3	±3	±3	±3	±3	±3	±3	±3	±3	±3	±7	±3	±7	±3	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7		
Drug administration training [e]		X																													
Study drug observer training [f]																															
12 lead ECG		X																													X
Physical examination [g]		X	X	X	X	X	X	X	X	X		X		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Weight		X	X	X	X	X	X	X	X	X		X		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Height		X				X				X		X		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
Vital signs		X	X	X	X	X	X	X	X	X		X		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Clinical safety laboratory tests [h]		X	X	X	X	X	X	X	X	X		X		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
C-reactive protein		X								X				X			X		X		X		X		X		X		X	X	X
Erythrocyte sedimentation rate [i]		X																													X
Serum cortisol [j]		X															X						X						X		X
Complements C3 and C4		X				X				X		X		X		X	X		X		X		X		X		X		X	X	X
Tryptase		X																												X	
Urine albumin/creatinine ratio [k]		X								X				X			X		X		X		X		X		X		X	X	X
Urine N-methylhistamine		X																												X	
Urine pregnancy test [l]		X	X	X	X	X	X	X	X	X		X		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
3-day diet diary [m]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
PK (plasma pegvaliase) [n]		X				X				X		X		X		X	X		X		X		X		X		X		X		X

Assessment [a]	Study Week	73[b]	74	75	76	77	78	79	80	81	83	85	87	89	91	93	97	101	105	109	113	117	121	125	129	133	137	141	145	HRV[c]	SC/ET[d]
	Study Day	505	512	519	526	533	540	547	554	561	575	589	603	617	631	645	673	701	729	757	785	813	841	869	897	925	953	981	1009		
	Window (Days)	±3	±3	±3	±3	±3	±3	±3	±3	±3	±3	±7	±3	±7	±3	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7		
Blood for exploratory analyses [o]		X				X				X		X		X		X	X	X	X	X	X	X	X	X	X	X	X	X		X
Blood Phe, tyrosine [p]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Immunogenicity assessments [q]		X	X	X	X	X	X	X	X	X		X		X		X	X		X		X		X		X		X		X	X	X
ADHD RS-IV [r]		X										X					X			X			X			X			X		X
BRIEF2 [r]		X										X					X			X			X			X			X		X
PKU-SSIS [s]		X										X					X			X			X			X			X		X
PGIS [s]		X										X					X			X			X			X			X		X
PGIC [s]		X																		X									X		X
CaGIS [r]		X										X					X			X			X			X			X		X
CaGIC [r]		X																		X									X		X
CGIS [t]		X										X					X			X			X			X			X		X
Weekly Phone Call (non-visit weeks) [u]																															
Adverse events [v]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant medication [v]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Administer study drug [w]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		

ADHD RS-IV, attention deficit hyperactivity disorder rating scale IV; BRIEF2, Behavior Rating Inventory of Executive Function, Second Edition; ECG, electrocardiogram; eCRF, electronic Case Report Form; HRV, hypersensitivity reaction visit; NSAID, non-steroidal anti-inflammatory drug; PE, physical examination; PK, pharmacokinetics; PKU, phenylketonuria; SC/ET, Study Completion/Early Termination visit.

[a] Scheduled visits take place at the hospital setting (for all dosing visits for the first 8 weeks, and for all dose escalation visits), clinic, or, with the approval of the investigator or designee, virtually. For virtual visits the investigator/designee performs assessments while online with the participant, apart from specimen collections and vital signs, which are performed by home healthcare personnel. Non-clinic visits are via telephone. Assessments are performed pre-dose unless otherwise specified.

[b] For Cohort B control participants, the Week 73 visit marks both the end of Part 1, when their PKU is treated with diet alone, and the beginning of Part 2, when they receive pegvaliase. The Week 73 pre-dose assessments constituting the end of Part 1 are shown in the Week 73 column of Table 1.3.4. These same assessments, used as baseline assessments for Part 2, are shown in the Week 73 column of Table 1.3.5, along with the administration of study drug that marks the beginning of Part 2.

[c] If the investigator determines that the NCI-CTCAE Grade ≥ 3 hypersensitivity reaction is related to administration with study drug, the participant will be asked to return to the clinic within 24 hours of event onset for a hypersensitivity reaction visit (HRV). Sampling for anti-pegvaliase IgE should be performed 8 to 24 hours after event onset and before the next dose of study drug.

[d] Participants should perform the Study Completion (SC) Visit 4 weeks after the last dose of study drug or after completion of study participation, whichever occurs last. For participants who terminate from the study early, the Early Termination (ET) Visit occurs within 4 weeks of the last study drug dose or after withdrawal from study participation, whichever occurs last. Participants who discontinue from study drug early will be asked to continue to complete the remaining study assessments until study completion as long as such continued participation will not detrimentally affect the health, safety, and welfare of the participant per investigator determination.

[e] Once participant/caregiver competence is documented, the participant/caregiver administers study drug daily.

[f] Must be completed prior to administration of the first dose of study drug. Additional observers identified after screening must be trained.

[g] A full PE will be performed at Week 73, Week 145, and the SC/ET visit. A brief PE will be performed at other visits. A full physical examination will include, at a minimum, general appearance (head, eyes, ears, nose, and throat), and an assessment of cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, musculoskeletal, and neurologic systems. A brief physical examination may be performed at the discretion of the investigator based on the participant's clinical condition and may include, but is not limited to, general appearance and an assessment of cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, musculoskeletal, and neurologic systems.

[h] It is recommended that urine samples are obtained as a first or second morning void. In the event of elevated urinary protein on a test result, a repeat urinalysis should be performed. This repeat urine sample must be performed in the morning at the first or second morning void to allow for accurate test results and may be performed by a home healthcare nurse.

[i] To be done by a local laboratory.

[j] Serum cortisol samples should be taken before study drug administration in the morning before 10:00 am. A home healthcare nurse may collect samples for participant convenience. If 2 results are low and abnormal, the participant will be asked to perform additional sampling for serum cortisol and a low-dose conventional ACTH stimulation test or the ACTH test method based on the clinical practice at the site.

[k] It is recommended that urine samples be obtained as a first or second morning void. Participants with a confirmed urine/albumin creatinine ratio of ≥ 100 mg/g should be referred to a nephrologist for consultation if results were within normal range at Baseline. Participants who had elevated results at Baseline followed by a confirmed subsequent increase of 100-200 mg/g from Baseline should also be referred to a nephrologist for consultation.

[l] To be done locally (if applicable). If urine pregnancy test is positive or equivocal, serum pregnancy test (central laboratory) must be done.

[m] Participants should record at home all food, beverages, special low-protein foods, and medical foods consumed during the 3 consecutive days preceding a study visit. No change in protein (dietary and medical food) intake is allowed in Part 1.

[n] Blood is collected before study drug administration (pre-dose) within 2 hours of dosing. Intensive PK sampling will be performed at Week 145 and at dose escalation visits in all participants. Samples will be taken at pre-dose (within 2 hours of dosing); at 2, 4, 8, and 12 hours (± 30 minutes) post-dose; and 24 hours (± 2 hours) post dose. The 24-hour sample will be taken prior to the next daily dose.

[o] Sample collection should be performed where allowed by country-specific regulations and EC/IRB.

[p] Blood is collected for blood Phe analysis after fasting a minimum of 2.5 hours. In addition to the blood draws for blood Phe analysis collected in the clinic and shown in the SoA, there are 5 required blood collections that may be drawn either at the clinic or elsewhere (eg, at home). These are to occur at Weeks 83, 87, and 91, and 2 weeks prior to the planned escalations to 40 and 60 mg/day. Diet diary data collection must be done prior to all Phe collections.

[q] Immunogenicity assays include total anti-pegvaliase antibodies (TAb), anti-PAL IgG, anti-PAL IgM, anti-PEG IgM, anti-PEG IgG, and neutralizing antibodies (NAb). At the HRV, only anti-pegvaliase IgE will be assessed.

[r] Completed by parent/guardian. Assessments should be completed before other study visit assessments (eg, vital signs, blood draw for blood Phe, and study drug administration).

[s] Completed by participant. Assessments should be completed before other study visit assessments (eg, vital signs, blood draw for blood Phe, and study drug administration).

[t] Completed by the investigator or designee clinician. Assessments should be completed before other study visit assessments (eg, vital signs, blood draw for blood Phe, and study drug administration). Visits where CGIS will be assessed should take place in the hospital setting or clinic.

[u] Between scheduled clinic visits, the clinic staff will contact the participant weekly to monitor if the participant is experiencing problems with drug administration, to ask about any AEs or concomitant medications, and to answer questions.

[v] AEs and concomitant medications should be noted whenever a participant is assessed by study personnel. Beginning with start of study treatment, All AEs and SAEs are collected until 4 weeks after the last study drug dose or the Study Completion Visit/Early Termination visit, whichever occurs last. If there is a severe (Grade 3) injection site reaction, the participant may be referred to a dermatologist for consultation and a skin biopsy (optional; if clinically indicated). If photographs of the severe injection site reaction are taken, they may be collected by the sponsor.

[w] Participants must be premedicated with an H1 antagonist, an H2 antagonist, and an antipyretic approximately 2 to 3 hours prior to each dose of study drug until they reach the Maintenance Phase, defined as when participants achieve blood Phe ≤ 600 µmol/L while they are on a stable dose of pegvaliase. Premedication may be considered during the Maintenance Phase at the investigator's discretion, eg, approximately 2 to 3 hours prior to study drug for 1 week upon reintroduction of study drug, upon resolution of an AE, following any dose interruption of ≥ 4 days, and for any dose increases in the Extension Phase. Participants may also be premedicated at any other time in the study per investigator discretion. When an NSAID is administered as a premedication, it should be given with food. For non-clinic visits, participants are asked about study drug administration.

Table 1.3.6: Schedule of Activities for Part 2 (Extension Phase – Weeks 149-215)
(Pegvaliase Treatment for Participants Randomized to Diet-Only Treatment Arm)

Assessment [a]	Study Week	145[b]	149	153	157	161	165	169	173	177	181	185	189	193	197	201	205	209	213	215	HRV[c]	SC/ET[d]
	Study Day	1009	1037	1065	1093	1121	1149	1177	1205	1233	1261	1289	1317	1345	1373	1401	1429	1457	1485	1499		
	Window (Days)	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7		
Study drug observer training [e]																						
12 lead ECG																						X
Physical examination [f]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Weight	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Height	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
Vital signs	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Clinical safety laboratory tests [g]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
C-reactive protein	X		X		X		X		X		X		X		X		X		X	X	X	X
Erythrocyte sedimentation rate [h]																						X
Serum cortisol [i]	X						X						X						X			X
Complements C3 and C4	X		X		X		X		X		X		X		X		X		X	X	X	X
Tryptase																					X	
Urine albumin/creatinine ratio [j]	X		X		X		X		X		X		X		X		X		X	X	X	X
Urine N-methylhistamine																					X	
Urine pregnancy test [k]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
3-day diet diary [l]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
PK (plasma pegvaliase) [m]	X		X		X		X		X		X		X		X		X		X			X
Blood for exploratory analyses [n]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
Blood Phe, tyrosine [o]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Immunogenicity assessments [p]	X		X		X		X		X		X		X		X		X		X	X	X	X
ADHD RS-IV [q]	X			X			X			X			X			X			X			X
BRIEF2 [q]	X			X			X			X			X			X			X			X
PKU-SSIS [r]	X			X			X			X			X			X			X			X

Assessment [a]	Study Week	145[b]	149	153	157	161	165	169	173	177	181	185	189	193	197	201	205	209	213	215	HRV[c]	SC/ET[d]
	Study Day	1009	1037	1065	1093	1121	1149	1177	1205	1233	1261	1289	1317	1345	1373	1401	1429	1457	1485	1499		
	Window (Days)	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7		
PGIS [r]		X			X			X			X			X			X			X		X
PGIC [r]		X									X									X		X
CaGIS [q]		X			X			X			X			X			X			X		X
CaGIC [q]		X									X									X		X
CGIS [s]		X			X			X			X			X			X			X		X
Weekly Phone Call (non-visit weeks) [t]																						
Adverse events [u]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant medication [u]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Administer study drug [v]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		

ADHD RS-IV, attention deficit hyperactivity disorder rating scale IV; BRIEF2, Behavior Rating Inventory of Executive Function, Second Edition; ECG, electrocardiogram; eCRF, electronic Case Report Form; HRV, hypersensitivity reaction visit; NSAID, non-steroidal anti-inflammatory drug; PE, physical examination; PK, pharmacokinetics; PKU, phenylketonuria; SC/ET, Study Completion/Early Termination visit.

[a] Scheduled visits take place at the hospital setting (for all dosing visits for the first 8 weeks, and for all dose escalation visits), clinic, or, with the approval of the investigator or designee, virtually. For virtual visits the investigator/designee performs assessments while online with the participant, apart from specimen collections and vital signs, which are performed by home healthcare personnel. Non-clinic visits are via telephone. Assessments are performed pre-dose unless otherwise specified.

[b] The Week 145 visit marks both the end of the Primary Treatment Phase and the beginning of the Extension Phase. The pre-dose assessments constituting the end of the Primary Treatment Phase and the administration of study drug constituting the beginning of the Extension Phase are shown in the Week 73 columns of both Table 1.3.5 and Table 1.3.6.

[c] If the investigator determines that the NCI-CTCAE Grade ≥ 3 hypersensitivity reaction is related to administration with study drug, the participant will be asked to return to the clinic within 24 hours of event onset for a hypersensitivity reaction visit (HRV). Sampling for anti-pegvaliase IgE should be performed 8 to 24 hours after event onset and before the next dose of study drug.

[d] Participants should perform the Study Completion (SC) Visit 4 weeks after the last dose of study drug or after completion of study participation, whichever occurs last. For participants who terminate from the study early, the Early Termination (ET) Visit occurs within 4 weeks of the last study drug dose or after withdrawal from study participation, whichever occurs last. Participants who discontinue from study drug early will be asked to continue to complete the remaining study assessments until study completion as long as such continued participation will not detrimentally affect the health, safety, and welfare of the participant per investigator determination.

[e] Additional observers identified after screening must be trained.

[f] A full PE will be performed at the SC/ET visit. A brief PE will be performed at other visits. A full physical examination will include, at a minimum, general appearance (head, eyes, ears, nose, and throat), and an assessment of cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, musculoskeletal, and neurologic systems. A brief physical examination may be performed at the discretion of the investigator based on the participant's clinical condition and may include, but is not limited to, general appearance and an assessment of cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, musculoskeletal, and neurologic systems.

[g] It is recommended that urine samples are obtained as a first or second morning void. In the event of elevated urinary protein on a test result, a repeat urinalysis should be performed. This repeat urine sample must be performed in the morning at the first or second morning void to allow for accurate test results and may be performed by a home healthcare nurse.

[h] To be done by a local laboratory.

[i] Serum cortisol samples should be taken before study drug administration in the morning before 10:00 am. A home healthcare nurse may collect samples for participant convenience. If 2 results are low and abnormal, the participant will be asked to perform additional sampling for serum cortisol and a low-dose conventional ACTH stimulation test or the ACTH test method based on the clinical practice at the site.

[j] It is recommended that urine samples be obtained as a first or second morning void. Participants with a confirmed urine/albumin creatinine ratio of ≥ 100 mg/g should be referred to a nephrologist for consultation if results were within normal range at Baseline. Participants who had elevated results at Baseline followed by a confirmed subsequent increase of 100-200 mg/g from Baseline should also be referred to a nephrologist for consultation.

[k] To be done locally (if applicable). If urine pregnancy test is positive or equivocal, serum pregnancy test (central laboratory) must be done.

[l] Participants should record at home all food, beverages, special low-protein foods, and medical foods consumed during the 3 consecutive days preceding a study visit. No change in protein (dietary and medical food) intake is allowed in Part 1.

[m] Blood is collected before study drug administration (pre-dose) within 2 hours of dosing. Intensive PK sampling will be performed at Week 145 and at dose escalation visits in all participants. Samples will be taken at pre-dose (within 2 hours of dosing), at 2, 4, 8, and 12 hours (± 30 minutes) post-dose; and 24 hours (± 2 hours) post dose. The 24-hour sample will be taken prior to the next daily dose.

[n] Sample collection should be performed where allowed by country-specific regulations and EC/IRB.

[o] Blood is collected for blood Phe analysis after fasting a minimum of 2.5 hours. In addition to the blood draws for blood Phe analysis collected in the clinic and shown in the SoA, there are 5 required blood collections that may be drawn either at the clinic or elsewhere (eg, at home). These are to occur at Weeks 83, 87, and 91, and 2 weeks prior to the planned escalations to 40 and 60 mg/day. Diet diary data collection must be done prior to all Phe collections.

[p] Immunogenicity assays include total anti-pegvaliase antibodies (TAb), anti-PAL IgG, anti-PAL IgM, anti-PEG IgM, anti-PEG IgG, and neutralizing antibodies (NAb). At the HRV, only anti-pegvaliase IgE will be assessed.

[q] Completed by parent/guardian. Assessments should be completed before other study visit assessments (e.g., vital signs, blood draw for blood Phe, and study drug administration).

[r] Completed by participant. Assessments should be completed before other study visit assessments (e.g., vital signs, blood draw for blood Phe, and study drug administration).

[s] Completed by the investigator or designee clinician. Assessments should be completed before other study visit assessments (e.g., vital signs, blood draw for blood Phe, and study drug administration). Visits where CGIS will be assessed should take place in the hospital setting or clinic.

[t] Between scheduled visits, the clinic staff will contact the participant weekly to monitor if the participant is experiencing problems with drug administration, to ask about any AEs or concomitant medications, and to answer questions.

[u] AEs and concomitant medications should be noted whenever a participant is assessed by study personnel. Beginning with start of study treatment, All AEs and SAEs are collected until 4 weeks after the last study drug dose or the Study Completion Visit/Early Termination visit, whichever occurs last. If there is a severe (Grade 3) injection site reaction, the participant may be referred to a dermatologist for consultation and a skin biopsy (optional; if clinically indicated). If photographs of the severe injection site reaction are taken, they may be collected by the sponsor.

[v] Participants must be premedicated with an H1 antagonist, and an H2 antagonist, and an antipyretic approximately 2 to 3 hours prior to each dose of study drug until they reach the Maintenance Phase, defined as when participants achieve blood Phe ≤ 600 µmol/L while they are on a stable dose of pegvaliase. Premedication may be considered during the Maintenance Phase at the investigator's discretion, eg, approximately 2 to 3 hours prior to study drug for 1 week upon reintroduction of study drug, upon resolution of an AE, following any dose interruption of ≥ 4 days, and for any dose increases in the Extension Phase. Participants may also be premedicated at any other time in the study per investigator discretion. When an NSAID is administered as a premedication, it should be given with food. For non-clinic visits, participants are asked about study drug administration.

2 INTRODUCTION

Phenylketonuria (PKU, OMIM 261600) is a rare autosomal recessive genetic disorder that is characterized by an inability of the body to break down the amino acid, phenylalanine (Phe), and is caused by mutations in the gene encoding phenylalanine hydroxylase (PAH). PAH catalyzes the conversion of the essential amino acid Phe to tyrosine, and this enzymatic activity is facilitated by tetrahydrobiopterin (BH4). PAH deficiency results in abnormally elevated concentrations of Phe. The estimated incidence of PKU is 1 in 15,000 births in the United States (US) (NIHCDP 2001).

The pathophysiology of PKU is well understood. A substantial body of evidence confirms that elevated Phe is directly toxic to cells in the brain, where it causes inhibition of protein synthesis in humans and animals (Wall 1990), affects the normal morphology of myelinating proteins (Dyer 1996), and leads to arrested or delayed development of dendrites and synapses in the cerebral cortex (Huttenlocher 2000). Neuropathology in PKU has been shown to derive from oxidative stress, impaired neurotransmitter metabolism, impaired protein synthesis and lipid metabolism, and brain energy metabolism alterations (Schuck 2015). The neurotoxicity of elevated Phe is a direct effect of Phe itself (Kaufman 1989). No abnormalities in Phe metabolites are observed in patients with PKU and normal Phe metabolites are toxic only at concentrations that are much higher than those reported in individuals with PKU.

With newborn screening followed by Medical Nutritional Therapy (MNT), the most severe manifestation of the disease, mental retardation, is prevented. Phe restriction with MNT involves consumption of semisynthetic medical foods that provide a source of Phe-free protein, modified low-protein foods, a small amount of natural protein (to provide required amounts of Phe), and other supplements (eg, vitamins and minerals) to correct nutritional deficiencies that can result from Phe restriction. Without highly restricted Phe intake and MNT, individuals with PKU quickly reach and sustain toxic concentrations of blood Phe. Individuals with classic PKU may have a complete absence or profound deficiency of PAH and typically show very high elevations of blood Phe (≥ 1200 µmol/L).

Historically, it was recommended that adherence to the Phe-restricted diet could end after age 6, but later studies have shown that patients who maintained Phe control longer had better outcomes in neuropsychological performance and areas of attention and speed of processing (Cerone 1999; Channon 2007; Rey 1996). A robust body of research suggests that early blood Phe control through dietary management is imperative in PKU populations, and that adult intelligence quotient (IQ) levels are correlated with the duration and vigilance of dietary management in childhood and adolescence (Pietz 1997; Smith 2000; Stemerdink 2000; Waisbren 1999).

To be optimally effective, a PKU diet needs to be started immediately after birth and continued for life. The American College of Medical Genetics and Genomics (ACMG) practice guidelines for PKU recommend life-long management and maintenance of metabolic control as essential to optimal functioning of individuals with PKU, with a goal of maintaining blood Phe concentrations ≤ 360 µmol/L (Vockley 2014). Similar European guidelines for the management of PKU recommend treatment target Phe levels of ≤ 600 µmol/L for patients older than 12 years (van Spronsen 2017).

Compliance with diet is a significant component of disease management, but life-long dietary compliance can be extremely difficult. A study conducted over 6 years at 4 treatment centers showed that dietary compliance in PKU populations was found to decline with age. Specifically, during infancy and in pre-school aged children, compliance with restrictive PKU diet is high because their diet is carefully managed by a parent and nutritionist. However, when PKU children start school, their blood Phe levels increase because of changes in socialization and changes in their palate, making compliance to a Phe-restricted diet more challenging (Walter 2002; Walter 2004). As children age, they become more likely to find formula and synthetic low-Phe foods distasteful and they become exposed to a broader range of food and peer pressure, both of which encourage noncompliance (MacDonald 2010). Adolescents/young adults with PKU are at particular risk for non-compliance with MNT as they transition away from childhood when parental oversight of dietary control decreases (Brown 2016). In addition, adolescents/young adults who have stopped MNT and attempt to resume a Phe-restricted diet into adulthood have difficulty maintaining the diet, with 31% of patients no longer maintaining dietary control after 10 months of restarting (Schuett 1985).

Therefore, despite the best efforts of the medical community and patients, life-long therapy with MNT with Phe restriction is not sustainable nor feasible as a treatment option for the majority of patients with PKU (Walter 2002; Burton 2010; Aoki 2007). The inability to adhere to MNT results in uncontrolled blood Phe levels associated with executive dysfunction, depression, and a variety of behavioral and psychiatric problems (Moyle 2007; Pietz 1997; Smith 2000; Waisbren 1999). Adolescents with PKU with poor metabolic control represent a patient population in great need of additional therapeutic options.

BioMarin has developed pegvaliase as a subcutaneous enzyme substitution treatment to reduce blood Phe levels in adult patients with PKU. Enzyme replacement therapy with PAH is not feasible due to the instability of PAH in plasma and the difficulty with efficiently delivering an intact, active form of the enzyme to the liver (Harding 2003; Kim 2004). Pegvaliase is a genetically modified phenylalanine ammonia lyase (PAL) enzyme product of

the cyanobacterium *Anabaena variabilis*, which is PEGylated to decrease immunogenicity and increase half-life. Unlike PAH, the PAL enzyme is active in plasma and, independently of cofactor BH4 which is required for PAH activity, catalyzes Phe to trans-cinnamic acid (t-CA) and ammonia, which are metabolized in the liver and excreted in the urine, respectively. The pegvaliase mechanism of action corrects the underlying PAH deficiency that leads to inability to metabolize Phe, increased Phe levels in the body, and consequent neurocognitive, neuropsychiatric, and executive functioning deficits.

A comprehensive review of pegvaliase is contained in the Investigator's Brochure (IB). Investigators must review this document prior to initiating this study.

2.1 Study Rationale

Although it is recommended that adolescents with PKU maintain good metabolic control as they move into adulthood (Vockley 2014), in separate surveys > 60% of adolescents (13 to 17 years of age) in the US have blood Phe concentrations above recommended targets (Jurecki 2017) and 79% of patients 15 to 19 years report blood Phe concentrations above targets (Walter 2002). Adolescents aged 12 to 17 years with PKU report difficulties with maintaining blood Phe control as they transition away from childhood when parental oversight of dietary and blood Phe control decreases (Brown 2016; van Spronsen 2017). In a 2015 National PKU Alliance (NPKUA) survey conducted among over 625 adults and families, approximately 25% of those caregivers indicated that their children's average blood Phe levels were in excess of the ACMG-recommended targeted range (120 to 360 µmol/L), and over half of all respondents (adults and caregivers) reported that it was somewhat to very difficult to manage their PKU (Brown 2016).

Adolescent patients report difficulties adhering to a Phe-restricted diet to maintain blood Phe levels within a safe range (Brown 2016; van Spronsen 2017), and a clinic survey assessing compliance with clinical guidance and dietary restriction showed that 60% of adolescents aged 13 to 17 were non-compliant, with an associated increase of Phe levels to > 360 µmol/L in 50% of patients and Phe levels > 600 µmol/L in 22% of patients. The same survey demonstrated that non-compliance starts as early as ages 5 to 12, and non-adherence to guidelines increases with age.

For adolescent patients at increased risk for loss of metabolic control maintained during childhood, a variety of adverse neuropsychological deficits (including deficits in executive functioning and psychiatric symptoms, such as anxiety, depression, and phobias) develop later in life when there is relaxation of blood Phe control (Huijbregts 2018; Diamond 1997). Adolescent PKU patients with blood Phe levels ≤360 µmol/L have been shown to perform better at neuropsychological and neurocognitive tasks (Huijbregts 2002; Jahja 2014).

The approval of Palynziq addressed a previous unmet medical need, providing adult PKU patients with a treatment that substantially reduced blood Phe concentrations and resulted in the stabilization of Phe above and beyond diet. The reduction and stabilization of blood Phe were associated with improvement in unmanaged symptoms, including neurocognitive symptoms. There is potential for improvement in these outcomes for adolescents as well given the demonstrated efficacy in adults. Therefore, expansion of Palynziq use to include adolescent patients 12 to 17 years with uncontrolled blood Phe concentrations greater than 600 µmol/L on existing management would broaden the patient population who can benefit from Palynziq treatment and address a currently underserved patient population.

The pathogenesis and pathophysiology of PKU, driven by an absence or deficiency in PAH enzyme leading to high Phe concentration, is applicable to both adolescents and adults. The pegvaliase mechanism of action to correct the underlying metabolic deficiency of PAH gene is the same in both adults and adolescents. Adolescent PKU patients are not expected to have specific ongoing developmental changes that could result in a different safety or efficacy outcome with pegvaliase treatment.

2.2 Background

Palynziq received marketing authorization in the US and European Union (EU). The original Biologics License Application (BLA) for Palynziq was approved by the Food and Drug Administration (FDA) at dosages up to 40 mg/day on 24 May 2018, to reduce blood Phe concentrations in adult patients with phenylketonuria (PKU) who have uncontrolled blood Phe concentrations greater than 600 µmol/L on existing management (BLA STN 761079). A supplemental Biologics License Application (sBLA) that included the addition of the maximum maintenance dose of 60 mg/day and long-term safety and efficacy data for adults was approved by the FDA on 06 October 2020. A Risk Evaluation and Mitigation Strategy (REMS) is a required element of the Palynziq approval in the US. Palynziq was approved by the European Commission on 03 May 2019 at dosages up to 60 mg/day to reduce blood Phe concentrations in patients aged 16 years and older who have inadequate blood Phe control (defined as blood Phe levels greater than 600 µmol/L) despite prior management with available treatment options.

BioMarin recognizes that PKU is a serious genetic disease diagnosed at birth that requires strict management of blood Phe levels from birth and continuing through life. Now that a positive benefit to risk profile has been established in adults, BioMarin is proposing this pegvaliase clinical study in adolescent PKU patients (ages 12 to 17 years old (US), inclusive, and 12 to 15 years old (EU), inclusive).

2.3 Benefit/Risk Assessment

Regulatory approval of Palynziq in the US and the EU was based on demonstration of efficacy from the Phase 2 and Phase 3 data of the pegvaliase clinical development program. The approval of pegvaliase addressed a previous unmet medical need, providing adult PKU patients with a treatment that substantially reduced blood Phe concentrations and resulted in the stabilization of Phe above and beyond diet. This reduction and stabilization of blood Phe was also associated with improvement in unmanaged symptoms including neurocognitive symptoms. Given the demonstrated efficacy in adults, there is the potential for improvement in these outcomes for adolescents.

The important identified risks for pegvaliase include acute systemic hypersensitivity reaction (equivalent to anaphylaxis for the purpose of this protocol; see Section 8.3.8.1 for how this risk is defined), angioedema, serum sickness, hypophenylalaninemia, persistent arthralgia, and severe injection site reaction (see Section 8.3.8 for event definitions). These events have been effectively treated and managed in the clinical trial and post-marketing settings. Healthcare providers, patients, and observers are trained in the recognition and management of both an acute systemic hypersensitivity reaction or angioedema, including the use of injectable epinephrine (adrenaline) and the need to seek immediate medical assistance. Premedication is required during Induction/Titration Dosing to help mitigate the risk of hypersensitivity reactions, including acute systemic hypersensitivity reactions. While hypophenylalaninemia has not been reported frequently in the post-marketing setting, it was observed during the clinical studies, where it was managed with decreases in pegvaliase dosing and increases in dietary protein intake. Participants will undergo frequent blood Phe monitoring during induction and titration and throughout dosing and will be required to dose reduce with confirmed hypophenylalaninemia (ie, 2 consecutive blood Phe levels < 30 µmol/L).

The important potential risks of treatment with pegvaliase also include potential complications of immune complex formation resulting in end-organ damage, fetal developmental toxicity, and acute systemic hypersensitivity reaction in patients taking other concurrent injectables containing PEG. No evidence of immune complex-mediated end organ damage or any early clinical signs potentially related to immune complex mediated disease have been reported in the clinical trial or commercial setting. Concurrent injectables containing PEG are prohibited during study treatment with the exception of COVID vaccinations (see Section 6.8.1).

The potential risks are justified by the anticipated benefits that may be afforded to adolescent participants. Each participant will have a comprehensive plan that includes frequent

monitoring of adverse events, blood Phe levels, and pregnancy during the study. Safety will be assessed by adverse event reporting and clinical laboratory assessments.

More detailed information about the known and expected benefits and risks and reasonably expected adverse events of pegvaliase may be found in the IB.

2.3.1 Risk Assessment

Table 2.3.1.1: Risks Associated with Study Drug

Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Acute systemic hypersensitivity reaction	In the completed Phase 2 and Phase 3 clinical trials, 16 out of 285 (6%) participants experienced 25 episodes of acute systemic hypersensitivity reactions of any severity. Acute systemic hypersensitivity reactions were managed by administration of adrenaline (44%; 11/25 episodes), corticosteroids (56%; 14/25 episodes), antihistamines (56%; 14/25 episodes), and/or oxygen (8%; 2/25 episodes). Four out of 16 (1%; 4/285) participants experienced a total of 5 episodes of an acute systemic hypersensitivity reaction that were considered severe. The underlying mechanism for acute systemic hypersensitivity reaction observed in clinical trials was non-IgE mediated Type III (immune-complex mediated) hypersensitivity. Acute systemic hypersensitivity reaction was most frequent during the Induction/Titration Phase (5% of participants; 19 episodes over mean treatment duration of 13 months) and decreased in the Maintenance Phase (2% of participants; 6 episodes over a mean treatment duration of 33 months). The risk of an acute systemic hypersensitivity reaction occurring is 6-fold higher in the Induction/Titration Phase compared to the Maintenance Phase.	To mitigate the risk of an acute systemic hypersensitivity reaction, an adult observer is required for a minimum of 1 hour post injection and premedication including H1 and H2 antagonists and an antipyretic is required 2 to 3 hours before each injection until participants reach the Maintenance Phase.[a] Premedication may be considered during the Maintenance Phase at the investigator's discretion. Participants will be provided with 2 epinephrine injectors and instructed to carry both injectors with them at all times. Participants and adult observers will be trained to recognize the signs and symptoms of anaphylaxis and, if these reactions occur, to call for emergency medical support and administer an epinephrine injector. For the duration of the study, each participant will be contacted weekly by study site personnel to monitor for drug administration problems and adverse events (AEs). Risk mitigation measures also include dosing instructions for other hypersensitivity adverse events (Table 6.5.1.1).
Angioedema	In the completed Phase 2 and Phase 3 clinical trials, 21 out of 285 (7%) participants experienced 37 episodes of mild to moderate episodes of angioedema (symptoms include one or more of the following: pharyngeal edema, swollen tongue, lip swelling, mouth swelling,	To mitigate the risk of hypersensitivity reactions, premedication including H1 and H2 antagonists and an antipyretic is required 2 to 3 hours before each injection until participants reach the Maintenance Phase.[a] Premedication may be considered

Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
	eyelid edema and face edema) occurring independent of acute systemic hypersensitivity reaction. Angioedema was most frequent during the Induction/Titration Phase (6% of participants; 27 episodes over mean treatment duration of 13 months) and decreased in the Maintenance Phase (3% of participants; 10 episodes over mean treatment duration of 33 months). The risk of angioedema occurring is 4.5-fold higher in the Induction/Titration Phase compared to the Maintenance Phase.	during the maintenance phase at the investigator's discretion. An adult observer is required for a minimum of 1 hour post injection throughout the study. For the duration of the study, each participant will be contacted weekly by study site personnel to monitor for drug administration problems and AEs. Risk mitigation measures also include dosing instructions for hypersensitivity adverse events (Table 6.5.1.1).
Serum sickness	In the completed Phase 2 and Phase 3 clinical trials, serum sickness was reported in 7 out of 285 (2%) participants. Serum sickness was most frequent during the Induction/Titration Phase (2% of participants; 6 episodes over mean treatment duration of 13 months) and decreased in the Maintenance Phase (0.6% of participants; 1 episode over mean treatment duration of 33 months). The risk of serum sickness occurring is greater than 2-fold higher in the Induction/Titration Phase compared to the Maintenance Phase.	To mitigate the risk of hypersensitivity reactions, premedication including H1 and H2 antagonists and an antipyretic is required 2 to 3 hours before each injection until participants reach the Maintenance Phase of dosing.[a] Premedication may be considered during the Maintenance Phase at the investigator's discretion. An adult observer is required for a minimum of 1 hour post injection throughout the study. For the duration of the study, each participant will be contacted weekly by study site personnel to monitor for drug administration problems and AEs. Risk mitigation measures also include dosing instructions for hypersensitivity adverse events (Table 6.5.1.1).
Persistent arthralgia (> 6 month duration)	In the completed Phase 2 and Phase 3 clinical trials, 245 out of 285 (86%) participants experienced episodes consistent with arthralgia (including back pain, musculoskeletal pain, pain in extremity, and neck pain). Arthralgia occurred as early as the first dose and can occur at any time during treatment. Arthralgia was most frequent during the Induction/Titration Phase (79% of participants; 1269 episodes over mean treatment duration of 13 months) and decreased in the Maintenance Phase (67% of participants; 673 episodes over mean treatment duration of 33 months). The risk of arthralgia occurring is 3.1-fold higher in the Induction/Titration Phase compared to the Maintenance Phase.	To mitigate the risk of hypersensitivity reactions, premedication including H1 and H2 antagonists and an antipyretic is required 2 to 3 hours before each injection until participants reach the Maintenance Phase of dosing.[a] Premedication may be considered during the Maintenance Phase at the investigator's discretion. An adult observer is required for a minimum of 1 hour post injection throughout the study. For the duration of the study, each participant will be contacted weekly by study site personnel to monitor for drug administration problems and AEs.

Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
	Seven (7%) percent of the participants experienced persistent arthralgia (≥ 6 months). The risk of persistent arthralgia may have significant impact on quality of life. Severe arthralgia (severe pain limiting self-care activities of daily living) was experienced in 5% of participants. Dose was not changed for 96% of episodes and all persistent arthralgia episodes resolved without sequelae.	Risk mitigation measures also include dosing instructions for hypersensitivity adverse events (Table 6.5.1.1).
Severe injection site reaction	Injection site reaction (ISR) was reported in 266 out of 285 (93%) participants. The most common ISR (occurring in at least 10% of participants) was reaction, erythema, bruising, pruritus, pain, swelling, rash, induration, and urticaria. Injection site reaction was most frequent during the Induction/Titration Phase (90% of participants; 3908 episodes over mean treatment duration of 13 months) and decreased in the Maintenance Phase (66% of participants; 1226 episodes over mean treatment duration of 33 months). The risk of an ISR occurring is 5.2-fold higher in the Induction/Titration Phase compared to the Maintenance Phase. Three ISRs consistent with granulomatous skin lesions were reported (each reaction occurring in 1 patient): granulomatous dermatitis (occurred 15 months after pegvaliase treatment and lasted 16 days), xanthogranuloma (occurred 12 months after pegvaliase treatment and lasted 21 months), and necrobiosis lipoidica diabeticorum (occurred 9 months after pegvaliase treatment and lasted 9 months). Necrobiosis lipoidica diabeticorum was treated with steroid injections and was complicated by a *Pseudomonas* infection. All of these injection site reactions resolved.	To mitigate the risk of hypersensitivity reactions, premedication including H1 and H2 antagonists and an antipyretic is required 2 to 3 hours before each injection until participants reach the Maintenance Phase of dosing.[a] Premedication may be considered during the Maintenance Phase at the investigator's discretion. An adult observer is required for a minimum of 1 hour post injection throughout the study. For the duration of the study, each participant will be contacted weekly by study site personnel to monitor for drug administration problems and AEs. Risk mitigation measures also include dosing instructions for hypersensitivity adverse events (Table 6.5.1.1). Any participant who experiences a severe (Grade 3) skin reaction will be asked to consult with a dermatologist for diagnosis and, if clinically indicated, a skin biopsy will be recommended. A Skin Reaction eCRF must be completed.
Hypophenylalaninemia	In the completed Phase 2 and Phase 3 clinical trials, 46% of the participants developed hypophenylalaninemia (blood phenylalanine levels below 30 µmol/L on two consecutive measurements). The risk of hypophenylalaninemia occurring is 2.1-fold higher in the maintenance phase	Blood Phe levels will be frequently monitored during induction and titration dosing and regularly monitored during maintenance dosing in both the Primary Treatment Phase and the Extension Phase of the study. Risk mitigation measures, including protocol-required adjustments in

Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
	compared to the Induction/Titration phase. In the completed adult clinical studies, dose reductions were effective in managing hypophenylalaninemia in participants experiencing hypophenylalaninemia despite appropriate levels of protein intake.	the pegvaliase dose (with or without modifications to the dietary intake of Phe) will be implemented to mitigate the potential risk of chronic hypophenylalaninemia levels (< 30 µmol/L) in adolescent participants.
Complications of immune complex formation resulting in end-organ damage	No evidence from clinical trials or post-marketing reports of immune complex-mediated end organ damage or any early clinical signs potentially related to immune complex mediated disease.	Risk mitigation measures include monitoring clinical laboratory tests (chemistry, hematology, urinalysis) and adverse events for immune complex-mediated end-organ damage. These mitigations will also allow early detection of adverse effects of PEG accumulation. For the duration of the study, each participant will be contacted weekly by study site personnel to monitor for AEs.
Fetal development toxicity	There are limited data from the use of pegvaliase in pregnant women. Animal studies have shown maternal reproductive toxicity that was associated with decreased blood phenylalanine concentrations below normal levels. Uncontrolled blood phenylalanine levels (hyperphenylalaninemia) before and during pregnancy are associated with increased risk for miscarriage, major birth defects (including microcephaly and major cardiac malformations), intrauterine fetal growth retardation and future intellectual disability with low IQ. Additional risk to the unborn child due to hypophenylalaninemia is not established.	If sexually active, male or female participants must not plan to become pregnant (self or partner) and must use 2 acceptable methods of contraception while participating in the study beginning at Screening and for 4 weeks after discontinuing study drug. Contraceptive use must be consistent with the guidance provided in Appendix 4, Section 10.4 of this protocol.
Unpredictable immune-mediated response in patients < 16 years	The adult development program was restricted to participants aged > 16 years of age. Review of spontaneous adverse reaction reports in patients < 16 years of age, using Palynziq in the commercial setting has not identified any unpredictable immune responses. The adverse reactions reported in patients < 16 years of age are consistent with the known safety profile of Palynziq as presented in the USPI and SmPC.	To mitigate the risk of hypersensitivity reactions, premedication including H1 and H2 antagonists and an antipyretic is required 2 to 3 hours before each injection until participants reach the Maintenance Phase. [a] Premedication may be considered during the Maintenance Phase at the investigator's discretion. An adult observer is required for a minimum of 1 hour post injection throughout the study. For the duration of the study, each participant will be contacted weekly by study site personnel to

Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
		monitor for drug administration problems and AEs. Risk mitigation measures also include dosing instructions for hypersensitivity adverse events (Table 6.5.1.1).
Acute systemic hypersensitivity reactions in patients taking other concurrent injectables containing PEG	PEGylated proteins have the potential to elicit an immune response. Because antibodies bind to the PEG portion of pegvaliase, there may be potential for binding with other PEGylated therapeutics and increased hypersensitivity to other PEGylated injectables. In a single dose study of pegvaliase in adult participants with PKU, two patients receiving concomitant injections of medroxyprogesterone acetate suspension containing PEG experienced hypersensitivity reactions.	Risk mitigation measures include prohibiting any injectable drugs containing polyethylene glycol (PEG; other than pegvaliase), including medroxyprogesterone injection, within 3 months prior to the start of Screening/Run-in and during study participation with the exception of COVID vaccinations (see Section 6.8.1).

[a] Participants are in the Maintenance Phase when they achieve blood Phe ≤600 µmol/L while they are on a stable dose of pegvaliase. This is distinct from Maintenance Dosing, defined as the period following Induction/Titration Dosing when Maintenance Doses (20, 40, or 60 mg/day) are administered, as outlined in Table 6.5.1.

2.3.2 Benefit Assessment

The overall clinical benefit of pegvaliase was established in patients with PKU based on data from completed Phase 2 and Phase 3 clinical studies. The approval of Palynziq addressed a previous unmet medical need, providing adult PKU patients with a treatment that substantially reduced blood Phe concentrations and resulted in the stabilization of Phe above and beyond diet. This reduction and stabilization was also associated with improvement in unmanaged symptoms including neurocognitive symptoms. Given the demonstrated efficacy in adults, there is the potential for improvement in these outcomes for adolescents.

2.3.3 Overall Benefit/Risk Conclusion

Results from the robust pegvaliase clinical development program demonstrated that pegvaliase treatment up to 60 mg/day demonstrated a positive benefit-risk profile in patients with PKU with uncontrolled blood Phe levels > 600 µmol/L.

Taking into account the measures taken to minimize risk to participants participating in this study, the anticipated benefits that may be afforded to adolescent PKU patients treated with pegvaliase outweigh the potential risks.

3 OBJECTIVES AND ENDPOINTS

The primary, secondary, and exploratory objectives and associated endpoints are shown in Table 3.1.

Table 3.1: Objectives and Endpoints

Objectives	Endpoints
Primary	
To evaluate the efficacy of pegvaliase in adolescent participants with PKU	The primary efficacy endpoint is change in blood Phe concentration from treatment naïve baseline following 72 weeks on study. Change in blood Phe from baseline is compared between participants in the active (pegvaliase) and the control (diet-only) treatment arms (see Section 9.4.2).
To evaluate the safety of pegvaliase in adolescent participants with PKU	• Adverse events (AEs), including serious AEs (SAEs) • Clinical laboratory tests (chemistry, hematology, and urinalysis) results • Vital signs • Physical examination (including height, weight) • Electrocardiogram (ECG) test results • Immunogenicity test results (anti-PEG IgG, anti-PEG IgM, anti-PAL IgG, anti-PAL IgM, TAb, NAb, and anti-pegvaliase IgE [hypersensitivity reaction visits only]).
Secondary	
To characterize total dietary protein intake in adolescent participants with PKU after pegvaliase treatment	Total dietary protein intake must remain stable for change in blood Phe, the primary endpoint, to be accurately assessed. To gauge the stability of total protein intake, the change from baseline at Week 73 in total dietary protein intake (measured in grams/kilogram [g/kg]) (defined as the sum of the protein intake from medical food and intact food) will be measured (see Section 9.4.3).
To characterize the pharmacokinetics (PK) of pegvaliase in adolescent participants with PKU	Trough plasma PK samples will be collected and analyzed as specified in the SoA. PK parameters assessed with intensive PK sampling will include $AUC_{0\text{-}24hr,ss}$, $C_{max,ss}$, $T_{max,ss}$, $t_{1/2}$, $CL_{ss/F}$, and $V_{dz/F}$. For participants randomized to the active (pegvaliase) treatment arm, intensive PK sampling will be performed at Week 73 and for dose escalations to 20, 40, and 60 mg/day. For participants randomized to the control (diet-only) treatment arm, there are no PK assessments in Part 1. In Part 2, intensive PK sampling will be performed at Week 145 and for dose escalations to 20, 40, and 60 mg/day (see Section 8.4).

Objectives	Endpoints
Exploratory Objectives	
To evaluate the effect of pegvaliase treatment on neurocognitive outcomes in adolescent participants with PKU	• The ADHD Rating Scale IV inattention subscale (ADHD RS-IV) rated by the parent/guardian and performed at Baseline (Day 1, pre-dose) and every 12 weeks throughout the study (see Section 8.1.3.1.1). • The Behavior Rating Inventory of Executive Function, Second Edition (BRIEF2) rated by the parent/guardian and performed at Baseline (Day 1, pre-dose) and every 12 weeks throughout the study (see Section 8.1.3.1.2). • Change from baseline (or first administration) in Phenylketonuria Symptom Severity and Impacts Scale (PKU-SSIS) Version 2.0 scores.
To assess changes in PKU symptoms and impacts	• Change from baseline (or first administration) in global severity items (Patient Global Impression of Severity [PGIS], Caregiver Global Impression of Severity [CaGIS], and Clinician Global Impression of Severity [CGIS]). • Change in global change items (Patient Global Impression of Change [PGIC] and Caregiver Global Impression of Change [CaGIC]).
To compare participants' optimal maintenance pegvaliase dose across different age groups within the study	• Time to participants' optimal maintenance dose compared to older age subsets • Number of participants who require the highest (60 mg/day) maintenance dose • Percentage/number of participants in the blood Phe target ranges of < 600 µmol/L and < 360 µmol/L.
Explore the biochemical, molecular, and cellular aspects of PKU	Blood will be collected to evaluate biochemical, molecular, and cellular aspects of PKU and to develop the assays used for these evaluations.
PAH genotyping	Testing will be performed on samples collected Day 1 pre-dose where allowed by local health authorities and the EC/IRB.

4 STUDY DESIGN

4.1 Overall Design

This Phase 3 multi-center study is designed to evaluate the safety and efficacy of pegvaliase administered daily to adolescent participants (ages 12 to 17 years old (US), inclusive, and 12 to 15 years old (EU), inclusive) with PKU. Pegvaliase will be subcutaneously injected in a hospital setting for the first 8 weeks and thereafter at the clinic or at home by a trained participant, caregiver, adult observer, or visiting healthcare provider. Pegvaliase will also be injected in a hospital setting for any subsequent dose escalations. The study design is summarized in Figure 1.2.1.

The study will enroll approximately 54 US and EU participants (ages 12 to 17 years old (US), inclusive, and 12 to 15 years old (EU), inclusive) who will be evaluated using an open-label, 2-arm, randomized control design, with diet-only management of PKU as the control. Participants will be randomized in a 2:1 ratio to the active and control arms, respectively, with 36 participants receiving pegvaliase and 18 participants managing their PKU with diet alone.

The primary efficacy endpoint is the change from baseline in blood Phe following 72 weeks on study (the end of Part 1). Beginning at Week 73, participants in the active treatment arm will continue to receive pegvaliase in the Extension Phase (Part 2) for up to an additional 142 weeks (through Week 215) in order to evaluate the long-term safety and efficacy of pegvaliase in adolescent participants with PKU. Beginning Week 73, participants in the control treatment arm will initiate pegvaliase treatment and, from Week 73 through Week 215, will follow the same schedule for dosing, study visits, and assessments that the active arm participants followed.

Eligible participants are required to have 2 blood Phe concentration measurements > 600 µmol/L during the Screening/Run-in Period (7 to 10 days apart) and an average blood Phe concentration > 600 µmol/L over the past 12 months prior to Screening based on available historical data. Once enrolled, patients will be dosed with pegvaliase according to the I/T/M dosing regimen based on the US package insert (USPI), with the guidance to increase dose depending on individual blood Phe lowering efficacy (per investigator's discretion). Dose should be increased to 40 mg/day if blood Phe is > 360 µmol/L (recommended) after 24 weeks dosing at 20 mg/day, and increased to 60 mg/day if blood Phe is > 360 µmol/L (recommended) after 16 weeks dosing at 40 mg/day. If a participant's blood Phe is > 600 µmol/L after 16 weeks of continuous treatment with pegvaliase at 60 mg/day,

pegvaliase should be permanently discontinued. BioMarin recommends that the target blood Phe levels should be based on individual blood Phe lowering efficacy.

In Part 1 (through the Week 73 visit), randomized participants in both the control (diet-only) and the active (pegvaliase) treatment arms will be required to maintain the same level of dietary protein intake they established during Screening/Run-in. In Part 2, participants in the control (diet-only) arm must continue to maintain this prescribed level of protein intake, whereas participants randomized to the active (pegvaliase) arm whose blood Phe concentration is ≤ 360 µmol/L for a minimum of 4 weeks may adjust dietary protein intake based on individual participant response to pegvaliase and guidance from the investigator.

4.2 Scientific Rationale for Study Design

This Phase 3 multi-center study is designed to evaluate the safety and efficacy of pegvaliase administered daily to adolescent participants with PKU. Approximately 54 US and EU participants (ages 12 to 17 years old (US), inclusive, and 12 to 15 years old (EU), inclusive) will be evaluated using an open-label, 2-arm, randomized control design, with diet-only management of PKU as the control.

A RCT design, with diet-only management of PKU as the control, will allow for robust data collection and minimize the risk of confounding factors influencing the results. An open-label diet-only control arm is the best comparator for this age group as daily placebo injections would place undue burden on adolescent PKU patients.

Except for participants randomized to pegvaliase in Part 2 whose blood Phe concentration is ≤ 360 µmol/L for a minimum of 4 weeks, participants in both the control (diet-only) and active (pegvaliase) treatment arms are required to maintain a consistent level of protein intake throughout the study (see Section 4.1 and Section 8.1.2). Participants are required to maintain a consistent level of protein intake to ensure that the safety and efficacy endpoints are attributable to pegvaliase rather than to changes in dietary protein intake.

Pegvaliase will be subcutaneously injected in a hospital setting for the first 8 weeks and for all subsequent dose escalations, and afterwards at the clinic or at home by a trained participant, caregiver, adult observer, or visiting healthcare provider. Administration at home makes daily treatment less burdensome for participants, thereby improving treatment compliance.

An adult observer is required for a minimum of 1 hour post injection throughout the study. An adult observer is defined as someone who will be present with the participant during and after pegvaliase administration, is able to recognize the signs and symptoms of an anaphylaxis episode, and is able to call for emergency medical support and administer

epinephrine, if warranted. The requirement for an adult observer was added to ensure that participants self-administering pegvaliase would have help in recognizing and quickly responding to signs and symptoms of anaphylaxis should they emerge.

The primary efficacy endpoint is the change from baseline in blood Phe following 72 weeks on study (the end of Part 1). Blood Phe level is the established clinical marker used to diagnose PKU and assess disease control, and reduction of blood Phe concentration to target levels is the universally acknowledged treatment goal for PKU patients. Published literature support the clinical benefit of lowering blood Phe concentration to ≤ 600 µmol/L for associated improvement in clinical outcomes including cognitive performance, behavior and mood (Palermo 2017; Romani 2017; Burgard 1999; Azen 1996; Smith 1989; Fitzgerald 2000; Costello 1994; Fonnesbeck 2013; Gassio 2003).

In addition, evidence has shown improved clinical benefit with even more aggressive blood Phe lowering targets. In children and adolescents with PKU, studies show that patients with blood Phe levels < 360 µmol/L perform better at neuropsychological and neurocognitive tasks (Huijbregts 2002; Jahja 2014). The findings from these studies demonstrate the need to adequately control blood Phe levels in PKU pediatric patients to below the 600 µmol/L threshold.

4.2.1 Participant Input into Design

BioMarin sought input from the PKU community in designing this study. Parents and a scientist from the National PKU Alliance reviewed portions of the protocol and provided feedback. Among other important input, the community stressed the importance of:

- Setting expectations for study participation, something that is critical to parents during the informed consent process
- Ongoing support from site personnel during the study
- Training for adult observers
- Simplifying, as much as possible, the diet tracking requirements given the time commitment required on the part of families
- Flexibility in scheduling clinic visits and including home and virtual visits where possible

BioMarin has taken this PKU community feedback into account when designing the study:

- Detailed training will be provided for participants, parents, and adult observers, and will continue throughout the study.
- Diet diaries will be in paper format and, consistent with past studies, diet tracking is restricted to the 3 days prior to study visits.

- Scheduled visits will take place at the clinic or, with the approval of the investigator or designee, virtually. For virtual visits the investigator/designee performs assessments while online with the participant, apart from specimen collections and vital signs, which are performed by home healthcare personnel.

4.3 Justification for Dose and Dose Regimen

Pegvaliase is a daily treatment for patients with PKU that will be administered in an Induction/Titration/Maintenance (I/T/M) regimen described in Section 6.5. Previous clinical studies have suggested the primary clearance mechanism of pegvaliase from plasma is via formation of circulating immune complexes leading to complement activation and removal of the drug by phagocytosis, indicating pegvaliase plasma exposure was driven by immune response. Considering that the immune system matures prior to adolescence (Olin 2018; Georgountzou 2017), the immune response to pegvaliase among adolescent participants is expected to be similar to the responses observed in 16 to 57-year-old study participants in the pegvaliase clinical development program.

Following dosing in a hospital setting for the initial doses (first 8 weeks) and apart from subsequent dose escalations to 20, 40, and 60 mg/day, which will also be administered in a hospital setting, pegvaliase will typically be administered at home by the trained participant, though pegvaliase may be also administered at home by a trained caregiver or visiting healthcare provider. In addition to training in drug administration prior to the first in-clinic dose, participants, caregivers, and adult observers will receive extensive training on how to monitor and respond to possible AEs (including acute systemic hypersensitivity reaction [anaphylaxis]) that may be associated with study drug treatment.

Pegvaliase will be administered using an I/T/M dosing regimen similar to the regimen used in Phase 3 studies performed in the US (165-301 and 165-302) and included in the USPI and SmPC (see Table 6.5.1 and Figure 1.2.2). During pegvaliase clinical development, this dosing regimen was found to help mitigate the onset and severity of hypersensitivity reactions while substantially reducing blood Phe concentrations.

The pegvaliase dose levels in this study have been evaluated in 6 multiple-dose Phase 2 and Phase 3 studies. The dose level for induction in 165-306 will be 2.5 mg once weekly and was chosen because this dose with a once weekly dosing frequency administered during the initial 4 weeks of treatment (ie, induction) used in Studies PAL-002, 165-205, and 165-301 has been associated with a lower incidence and severity of hypersensitivity reactions when compared with 5 days per week dosing frequency at a higher starting dose (0.4 mg/kg) studied in Study PAL-004.

4.4 End of Study Definition

A participant is considered to have completed the study if he/she has completed all scheduled visits, including procedures scheduled at the Study Completion/Early Termination (SC/ET) visit, as shown in the SoA (see Section 1.3), or the study is closed by the sponsor. The end of the study is defined as the date of the last visit of the last participant in the study.

5 STUDY POPULATION

Prospective approval of protocol deviations to eligibility criteria, also known as protocol exemptions, is not permitted. Rescreening is allowed for out of range laboratory values.

5.1 Inclusion Criteria

Participants are eligible to be included in the study only if all of the following criteria apply:

Age

1. Is 12 to 17 years old (US), inclusive, or 12 to 15 years (EU), inclusive, at the start of the Screening/Run-in Period (Day -28).

Type of Participant and Disease Characteristics

2. Diagnosis of PKU and failure to maintain recommended blood Phe levels on existing management (defined as failed both sapropterin dihydrochloride treatment and Phe-restricted diet) demonstrated by 2 blood Phe concentration measurements > 600 µmol/L during the Screening/Run-in Period (7 to 10 days in between blood Phe assessments) and average blood Phe concentration > 600 µmol/L over the past 12 months (per available data).
3. Willing and able to maintain and adjust dietary and medical protein food intake according to the study protocol under the supervision of a study dietician or adequately trained designee per investigator discretion during study participation.
4. If on medication for ADHD, depression, or other psychiatric disorder, stable dose of medication for ≥ 8 weeks prior to enrollment and willing to maintain stable dose unless a change is medically indicated.
5. An adult (≥ 18 years of age) has been identified who is willing and competent to observe the participant during study drug administration and for a minimum of 1 hour following administration.
6. Able and willing to comply with all study procedures.
7. Generally in good health, as evidenced by physical examination, clinical laboratory evaluations (hematology, chemistry, and urinalysis), and electrocardiogram (ECG) tests performed at Screening.

Sex and Contraceptive/Barrier Requirement

8. Males and females are eligible to participate in this clinical study.
9. Females with childbearing potential must have a negative pregnancy test at Screening/Run-in and be willing to have additional pregnancy tests during the study.
10. If sexually active, male or female participants must not plan to become pregnant (self or partner) and must use 2 acceptable methods of contraception while participating in the study beginning at Screening and for 4 weeks after discontinuing study drug.

Contraceptive use must be consistent with the guidance provided in Appendix 4, Section 10.4 of this protocol. Acceptable methods of contraception may include:

- Primary forms: hormonal (combination hormone-containing pills, patch, vaginal ring, or intrauterine device) or non-hormonal (copper-containing intrauterine device).
- Secondary forms: barrier forms and other forms of birth control and must include spermicide (eg, male condom; female condom is not an acceptable secondary form).

11. Contraceptive use should be consistent with local regulations regarding the methods of contraception for those participating in clinical studies.

Informed Consent

12. Participants must be capable of giving signed informed consent as described in Appendix 1, Section 10.1, which includes compliance with the requirements and restrictions listed in the informed consent form (ICF) and in this protocol. In the case of participants under the age defined by regional law or regulation, parent(s) or guardian(s) must be willing and able to provide written, signed informed consent after the nature of the study has been explained and prior to performance of any research-related procedure. Participants under the age of majority must be willing and able to provide written assent (if required by local regulations or the IRB/IEC) after the nature of the study has been explained and prior to performance of any research-related procedure.

5.2 Exclusion Criteria

Participants are excluded from the study if any of the following criteria apply:

Medical Conditions:

1. Concurrent disease or condition that would interfere with study participation or safety (eg, history or presence of clinically significant cardiovascular, pulmonary, hepatic, renal, hematologic, gastrointestinal, endocrine, immunologic, dermatologic, neurological, oncologic, or psychiatric disease).

Prior/Concomitant Therapies:

2. Taking any of the prohibited medications outlined in Section 6.8.1.
3. Previous treatment with pegvaliase.
4. Use of any medication that is intended to treat PKU, including the use of large neutral amino acids, within 14 days prior to the administration of study drug on Day 1.
5. Use or planned use of any injectable drugs containing polyethylene glycol (PEG; other than pegvaliase), including medroxyprogesterone injection, within 3 months prior to the start of Screening/Run-in and during study participation with the exception of COVID-19 vaccinations (Section 6.8.1).

6. A history of organ transplantation or on chronic immunosuppressive therapy.

Prior/Concurrent Clinical Study Experience

7. Use of any investigational product or investigational medical device within 30 days prior to Screening/Run-in or requirement for any investigational agent prior to completion of all scheduled study assessments.

Diagnostic Assessments

8. A positive test for HIV antibody, hepatitis B surface antigen, or hepatitis C antibody.
9. Alanine aminotransferase (ALT) concentration > 2 × the upper limit of normal (ULN).
10. Creatinine > 1.5 × ULN.

Other Exclusions

11. A history of substance abuse (as defined by the American Psychiatric Association: Diagnostic and Statistical Manual of Mental Disorders [DSM]) in the past 12 months or current alcohol or drug abuse.
12. Pregnant or breastfeeding or planning to become pregnant (self or partner) or breastfeeding at any time during the study.
13. Major surgery planned during the study period.
14. Inability to identify and/or communicate to others that the participant is experiencing symptoms of potential anaphylaxis due to cognitive impairment or other reasons.
15. Unwillingness or inability of participant/caregiver to carry auto-injectable epinephrine with them at all times.
16. Any condition that, in the view of the investigator, places the participant at high risk of poor treatment compliance or terminating early from the study.

5.3 Lifestyle Considerations

5.3.1 Meals and Dietary Restrictions

Details of all PKU-specific dietary therapy (eg, Phe intake, amino-acid supplementation) must be recorded in the source document and electronic case report form (eCRF).

Dietary Instruction

Participants must maintain dietary Phe intake levels that are consistent with their baseline levels for the duration of the study unless clinically indicated, per dietician or adequately trained designee per investigator/designee discretion to support individual nutritional needs during study participation (refer to Section 8.1.2).

Fasting Requirements

Fasting (water only) is required for at least 2.5 hours prior to any blood Phe measurement.

Any time fasting is required for visit assessments, the fasting assessments should be completed and fasting stopped before clinical outcome assessments are performed. The fast can be broken with a snack or meal as preferred.

5.3.2 Activity Restrictions

Participants should abstain from strenuous exercise for 48 hours before each blood collection for clinical laboratory tests. Participants may participate in light recreational activities (such as watching television or reading) prior to study visits, as appropriate.

5.4 Screen Failures

Screen failures are defined as participants who assent to participate (and whose parents/guardians consent to their participation) in the clinical study but who are not subsequently enrolled. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and the occurrence of any serious adverse event (SAE).

Individuals who do not meet the criteria for participation in the study may be rescreened. Rescreened participants should be assigned the same participant number as for the initial screening. If eligibility criteria are met on retest within the screening window, the participant may be enrolled after confirmation by the medical monitor. Participants who do not meet eligibility criteria within the screening window, will have to perform all Screening Visit procedures and assessments when re-screened.

5.5 Study Stopping Criteria

The independent Data Monitoring Committee (DMC) will be responsible for performing a benefit to risk assessment of pegvaliase in this patient population on a regular (quarterly) and ad hoc basis during the study and for making recommendations to BioMarin with regards to study conduct. The DMC Chair will be informed of all participants meeting the Individual Stopping Criteria Evaluation for Adverse Events During Pegvaliase Treatment (Section 8.3.8.1.1).

6 STUDY TREATMENT

6.1 Study Treatment(s) Administered

Study treatment is defined as any investigational treatment(s), marketed product(s), placebo, or medical device(s) intended to be administered to a study participant according to the study protocol.

The investigational treatment is pegvaliase (recombinant *Anabaena variabilis* phenylalanine ammonia lyase-PEG). For subcutaneous administration, participants are supplied with prefilled syringes in 3 sizes. Sizes are 2.5 mg (0.5 mL of 5 mg/mL protein concentration), 10 mg (0.5 mL of 20 mg/mL protein concentration), and 20 mg (1.0 mL of 20 mg/mL protein concentration).

The excipients in this drug product are tromethamine, tromethamine-hydrochloride, sodium chloride, trans-cinnamic acid, and water for injection.

Study treatment details are provided in Table 6.1.1.

Table 6.1.1: Study Treatment

Treatment Name	pegvaliase
Type	drug
Dose Formulation	prefilled syringe
Unit Dose Strength	Each PFS is filled to deliver at least 0.5 or 1 mL of formulation containing 2.5, 10, or 20 mg of pegvaliase
Dosage Level(s)	2.5 mg, 5 mg , 10 mg, 20 mg, 40 mg, 60 mg
Route of Administration	subcutaneous injection
IMP and NIMP	IMP
Sourcing	provided centrally by the sponsor
Packaging and Labeling	Drug product packaging is identified with lot and identification numbers and provided in a box labeled with the study number and identified to be for investigational use.
Current/Former Name(s) or Alias(es)	pegvaliase, BMN 165, rAvPAL-PEG (PEGylated recombinant Anabaena variabilis phenylalanine ammonia lyase), Palynziq® (trade name)

IMP, investigational medical products; NIMP, non-investigational medical products; PFS, pre-filled syringe.

In addition to pegvaliase, treatment in this study includes a diet in which total protein intake (defined as the sum of protein intake from medical food and intact food) must remain stable and consistent with baseline levels. Specifically, to accurately assess the primary endpoint comparison between the active (pegvaliase) and control (diet-only) treatment arms on the

change from baseline in blood Phe concentration following 72 weeks on study, total protein intake must remain stable and consistent with baseline levels for participants in both treatment arms through Part 1. In Part 2, participants in the control arm must continue to maintain this prescribed level of protein intake, whereas participants randomized to pegvaliase whose blood Phe concentration is ≤ 360 µmol/L for a minimum of 4 weeks may adjust dietary protein intake based on individual participant response to pegvaliase and guidance from the investigator. Details on diet monitoring are provided in Section 8.1.2.

6.1.1 Additional Drug Administration Instructions

Each prefilled syringe is intended for a single subcutaneous injection. The recommended injections sites include the front middle of thighs and the abdomen at least 2 inches (5 centimeters) away from the navel. If a caregiver is giving the injection, the top of the buttocks and the back of the upper arms are also appropriate injections sites. The injection sites for administration of pegvaliase should alternate between doses. Pegvaliase must not be injected into moles, scars, birthmarks, bruises, rashes, or areas where the skin is hard, tender, red, damaged, burned, inflamed, or tattooed. If multiple injections are needed for a single dose, injections should be administered at the same time of day and injection sites should be at least 5 cm away from each other. Doses should not be divided over the course of the day.

A participant (or participant-designated caregiver) must meet all of the following criteria to be eligible to administer the drug:

- Has no known cognitive impairments that may increase the safety risk per investigator assessment
- Has no medical history or current medications that may compromise administration of pegvaliase per investigator assessment
- Has completed all required drug administration training and has demonstrated administration competency per investigator assessment, including how to prepare pegvaliase for administration and safely perform the injection

Refer to Section 6.5 for study drug administration requirements regarding the presence of an adult observer and for premedication requirements.

Following a dose interruption of ≥ 4 doses, the investigator should obtain approval from the medical monitor prior to the participant restarting pegvaliase. Dose regimens may be revised provided the investigator obtains approval from the medical monitor prior to starting an alternative dose regimen.

The suitability for self-administration of pegvaliase will be reassessed by the investigator if a participant experiences any issues with self-administration. In addition, if a participant has an

NCI-CTCAE grade 3 or higher AE during the study, the participant's suitability for self-administration of pegvaliase will be reassessed by the investigator.

Between scheduled clinic visits, the clinic staff will contact the participant weekly to monitor if the participant is experiencing problems with drug administration, for any AEs, and to answer questions throughout the duration of the study. The participant must perform the scheduled assessments and procedures in the clinic as outlined in the SoA (Section 1.3). Training materials and other information specific to the study site personnel will be provided in the study reference materials, which includes drug administration training materials and instruction on the following:

- How to prepare and safely perform the injections of pegvaliase
- How to receive, store, and return both used and unused pegvaliase
- How to safely use and dispose of prefilled syringes used for injections of pegvaliase
- How to use a prefilled syringe every time drug is administered
- How to care for the injection site after an injection of pegvaliase
- How to document each pegvaliase injection
- How to identify an AE and how to report this to the study site
- How and when to use epinephrine
- Whom to contact at the study site in case of an emergency

Participants will be provided with a workbook to document the date and time of pegvaliase injections, injection site, any suspected AEs, and other relevant information. Dosing in response to an AE should be determined by the investigator.

6.2 Preparation/Handling/Storage/Accountability

At the study site, all study drug must be stored at 2-8°C (36-46°F), under the conditions specified in the IB, and in a secure area accessible only to the designated pharmacists and clinical site personnel. All study drug must be stored and inventoried, and the inventories must be carefully and accurately documented according to applicable state, federal, and local regulations; ICH Good Clinical Practice (GCP); and study procedures. Information regarding storage of study drug in participants' homes is provided in the study training materials.

The investigator or designee must confirm appropriate temperature conditions have been maintained during transit for all study treatment received and that any discrepancies are reported and resolved before use of the study treatment.

Only participants enrolled in the study may receive study treatment, and only authorized site staff may supply study treatment. All study treatment must be stored in a secure, environmentally controlled and monitored (manual or automated) area in accordance with the labeled storage conditions, with access limited to the investigator and authorized site staff.

The investigator, institution, qualified pharmacist, or the head of the medical institution (where applicable) is responsible for study treatment accountability, reconciliation, and record maintenance (ie, receipt, reconciliation, and final disposition records).

Participants are provided instructions for returning used and unused study drug prefilled syringes and for proper disposal at each site visit.

Further guidance and information for the final disposition of unused study treatments are provided in the Pharmacy Manual.

6.3 Measures to Minimize Bias: Randomization and Blinding

This is an open-label study; however, the specific intervention to be taken by a participant will be assigned using an IVRS/IWRS. The site will contact the IVRS/IWRS prior to the start of study intervention administration for each participant. The site will record the intervention assignment on the applicable case report form, if required. Potential bias will be reduced by central randomization.

6.4 Study Treatment Compliance

When participants are dosed at the site, they will receive study treatment directly from the investigator or designee, under medical supervision. The date and time of each dose administered in the clinic will be recorded in the source documents and recorded in the eCRF. The dose of study treatment and study participant identification will be confirmed at the time of dosing by a member of the site staff other than the person administering the study treatment.

When participants, caregivers, or home health nurses administer study treatment(s) at home, the date, time, and quantity of each dose of study drug administered to each participant must be recorded by the participant, caregiver, or home health nurse in a workbook provided for the study. The site staff will collect the workbook at scheduled clinic visits and transcribe this information to the appropriate eCRF.

These data will be used to assess compliance. Deviation(s) from the prescribed dosage regimen should be recorded in the eCRF. Treatment start and stop dates, including dates for treatment delays and/or dose reductions, should also be recorded in the eCRF.

6.5 Dose Modification

Participants will receive pegvaliase at a concentration of 5.0 or 20.0 mg/mL. Pegvaliase will be administered subcutaneously at dose levels in the range of 2.5 to 60 mg. The minimum dose of pegvaliase is 2.5 mg/week. The maximum allowable daily dose of pegvaliase is 60 mg/day (for a maximum weekly total dose of 420 mg).

The duration of the Primary Treatment Phase (Part 1) is 72 weeks, during which pegvaliase treatment will be initiated for participants randomized to the active (pegvaliase) treatment arm using an I/T/M dosing regimen. The recommended dosing schedule for participants dosing with pegvaliase is shown in Table 6.5.1. Induction will consist of a 4-week period when participants randomized to the active treatment arm will receive pegvaliase subcutaneously with a PFS at a fixed dose of 2.5 mg/week and during which the dosing regimen will not be modified in response to blood Phe concentration. Once participants complete induction they will titrate up to a dose of 10 mg/day (investigators may, at their discretion, include dosing at a 5 mg/day dose level). The dosing frequency will be increased gradually during titration dosing to daily (7 days/week). During Maintenance Dosing the dose will be increased to 20 mg/day, 40 mg/day, and 60 mg/day depending upon individual blood Phe lowering efficacy (per the investigator's discretion). The dose should be increased to 40 mg/day if blood Phe is > 360 µmol/L (recommended) after 24 weeks dosing at 20 mg/day and increased to 60 mg/day if blood Phe is > 360 µmol/L (recommended) after 16 weeks dosing at 40 mg/day. If a participant's blood Phe is > 600 µmol/L after 16 weeks of continuous treatment with pegvaliase at 60 mg/day, pegvaliase should be permanently discontinued. BioMarin recommends that the target blood Phe levels should be based on individual needs, with a target blood Phe ≤ 600 µmol/L.

Duration between dose titration steps may be increased in response to AEs. Dose level reductions due to AEs or hypophenylalaninemia (confirmed with 2 consecutive blood Phe values < 30 µmol/L) may be made at any time during the study at the discretion of the investigator. Phe levels will be regularly monitored, and adjustments in the pegvaliase dose (with or without adjustments to the dietary intake of Phe) will be required to mitigate the potential risk of chronic hypophenylalaninemia levels (< 30 µmol/L) in adolescent participants.

Following completion of Part 1, participants randomized to the active (pegvaliase) treatment arm will enter the Extension Phase (Part 2), during which participants will continue dosing with pegvaliase at their optimal maintenance dose level for up to an additional 142 weeks in order to evaluate the long-term safety and efficacy of pegvaliase in adolescent participants with PKU.

Following completion of Part 1, participants randomized to the control (diet-only) treatment arm in Part 1 will repeat the Part 1 assessment schedule while initiating pegvaliase dosing from Weeks 73 through 215.

Table 6.5.1: Study Drug Dose Titration Schedule

Part 1 Dosing	Duration [a]	Dose (mg)	Frequency of Administration (Doses Per Week)
Induction	Week 1	2.5	1
	Week 2	2.5	1
	Week 3	2.5	1
	Week 4	2.5	1
Titration	Week 5	2.5	2
	Week 6	10	1
	Week 7	10	2
	Week 8	10	4
	Week 9	10	7
Maintenance	20 mg daily: 24 weeks [b] 40 mg daily: 16 weeks [b,c] 60 mg daily: maximum dose [c]	20	7
		40	7
		60	7

[a] Minimum duration prior to dose escalation. Dose escalation may be delayed depending on participant tolerability. Investigators may, at their discretion, include dosing at a 5 mg/day dose level as part of the titration from 2.5 mg/week up to 10 mg/day.

[b] Prior to initial dose escalation, the minimum amount of time at 20 mg/day is 24 weeks and the minimum amount of time at 40 mg/day is 16 weeks. The dose should be increased to 40 mg/day if blood Phe is > 360 µmol/L (recommend) after 24 weeks dosing at 20 mg/day, and increased to 60 mg/day if blood Phe is > 360 µmol/L (recommend) after 16 weeks dosing at 40 mg/day.

[c] If multiple injections are needed for a single dose, the injections should be administered at the same time of day and injection sites should be at least 5 cm away from each other. Doses should not be divided over the course of the day. If a participant's blood Phe is > 600 µmol/L after 16 weeks of continuous treatment with pegvaliase at 60 mg/day, pegvaliase should be permanently discontinued.

Following enrollment, participants (and an adult caregiver) will be trained on how to administer study drug at home. Pegvaliase dosing will be conducted in a hospital setting for at least the first 8 weeks including titration up to 10 mg once daily, and for all subsequent dose escalations. The participant (and the adult caregiver) must demonstrate competency with injecting study drug in order to be permitted to administer study drug at home. Dosing at home will not be allowed until the principal investigator has assessed the participant's and

adult observer's competency in proper study drug administration and recognition and appropriate management of the potential risks.

Throughout the study, an adult observer must be present during study drug administration and for a minimum of 1 hour following administration. An adult observer is defined as someone who will be present with the participant during and after pegvaliase administration, is able to recognize the signs and symptoms of an anaphylaxis episode, and is able to call for emergency medical support and administer epinephrine, if warranted. Administration of study drug may only be performed if this adult observer is present. Participants and the adult observers who are observing them during study drug administration will be provided with information and extensive training regarding how to recognize a possible allergic reaction, the severity of the reaction, and instructions on what to do if a reaction occurs. Home administration may not performed until the investigator has assessed the adult observer's competency in proper study drug administration and the recognition and appropriate management of potential risks.

Participants will be provided with 2 epinephrine injectors and are required to carry both injectors with them at all times. Participants and adult observers will be trained to recognize the signs and symptoms of anaphylaxis and, if these reactions occur, to call for emergency medical support and administer an epinephrine injector. For the duration of the study, each participant will be contacted weekly by study site personnel to monitor for administration problems and AEs (see Section 8.3.8.1 for details on the management of suspected anaphylaxis in this study).

All participants receiving pegvaliase must be premedicated with an H1 antagonist, and an H2 antagonist, and an antipyretic approximately 2 to 3 hours prior to each dose until they reach the Maintenance Phase, defined as when participants achieve blood Phe ≤600 µmol/L while they are on a stable dose of pegvaliase. Premedication may be considered during the Maintenance Phase at the investigator's discretion, eg, approximately 2 to 3 hours prior to study drug for 1 week upon reintroduction of study drug, upon resolution of an AE, following any dose interruption of ≥ 4 days, and for any dose increases in the Extension Phase. Participants may also be premedicated at any other time in the study per investigator discretion.

This premedication regimen was successfully implemented in the Phase 3 adult clinical program. Participants will be provided with a workbook to document the date and time of study drug injections, the injection site, premedication use, and suspected AEs.

If ≥ 4 consecutive doses are missed for reasons other than safety related issues, the investigator should obtain approval from the medical monitor prior to the participant resuming study drug.

6.5.1 Dosing Modifications in Response to Hypersensitivity Adverse Events

Dosing modifications in response to a hypersensitivity adverse event (HAE) depends on the NCI-CTCAE grade and suspected relationship study drug. Dosing instructions are provided in Table 6.5.1.1 (regardless of previous occurrence). Dosing modifications are per reported HAE, independent of prior reported HAEs.

Table 6.5.1.1: Dosing Instructions in Response to a Hypersensitivity Adverse Event

NCI-CTCAE Grade [a]	Related to Study Drug	Action with Study Drug			Individual Stopping Criteria Evaluation [d]	HRV Assessment [e]
		Maintain [b]	Reduce [c]	Interrupt [c]		
1	Yes or No	X	(X) Optional	(X) Optional		Investigator discretion
2	Yes or No	X	(X) Optional	(X) Optional		Investigator discretion
3	No				X Immediately consult with sponsor medical monitor	Investigator discretion
3 [d]	Yes			X	X Immediately consult with sponsor medical monitor	Yes (if within 24 hours of onset)
4 [d]	Yes or No			X	X Immediately consult with sponsor medical monitor	Yes (if within 24 hours of onset)

AE, adverse event; CTCAE, Common Terminology Criteria for Adverse Events, Version 5.0; HRV, Hypersensitivity Reaction Visit; NCI, National Cancer Institute.

[a] NCI-CTCAE grade determination is performed by the investigator and may be done either via telephone or clinic visit.

[b] The investigator will instruct the participant to maintain the study drug dose at the time of AE onset until improvement to Grade 1 or resolution (per investigator assessment in the clinic or via telephone).

[c] The study drug dose may be reduced or interrupted if necessary per investigator determination.

[d] If a participant has an NCI-CTCAE Grade ≥ 3 hypersensitivity AE that is related to study drug and is suspected to meet Brown's criteria for severe (Grade 3) in the judgment of the investigator and/or the sponsor's medical monitor, the participant will be permanently discontinued from study drug.

[e] If the investigator determines that the NCI-CTCAE Grade ≥ 3 hypersensitivity reaction is related to administration with study drug, the participant will be asked to return to the clinic within 24 hours of event onset for evaluation, including laboratory tests (chemistry, hematology, urinalysis, anti-pegvaliase IgE [sampling should be performed 8 to 24 hours after event onset and before the next dose of study drug], urine albumin/creatinine ratio, CRP, C3, and C4).

Once an HAE (other than an anaphylaxis episode) improves to Grade 1 or resolves, the study drug dose may be increased, maintained, or reduced, at the discretion of the investigator. If reduced, reductions should be from 60 to 40 mg/day, from 40 to 20 mg/day, or from 20 to 10 mg/day, whichever is appropriate.

6.6 Treatment after the End of the Study

There is no treatment following completion of the study.

6.7 Overdose

For this study, any dose of pegvaliase greater than the prescribed dose will be considered an overdose.

BioMarin does not recommend specific treatment for an overdose. In the event of an overdose, the investigator should contact the BioMarin medical monitor immediately.

6.8 Concomitant Therapy

Any medication or vaccine (including over-the-counter or prescription medicines, vitamins, and/or herbal supplements) that the participant is receiving at the time of enrollment or receives during the study must be recorded on the designated eCRF along with:

- Reason for use
- Dates of administration including start and end dates
- Dosage information including dose and frequency

The medical monitor should be contacted if there are any questions regarding concomitant or prior therapy. The investigator may prescribe additional medications, deemed necessary to provide adequate prophylactic or supportive care, during the study, as long as the prescribed medication is not prohibited by the protocol (refer to Section 5.2). In the event of an emergency, any needed medications may be prescribed without prior approval, but the medical monitor must be notified of the use of any contraindicated medications immediately thereafter. Any concomitant medications added or discontinued during the study should be recorded on the eCRF. Medications should, whenever possible, not be recorded in the electronic database with a frequency of PRN.

6.8.1 Excluded Concomitant Medications

The following medications are prohibited starting at the time specified and until the end of the study:

- Any medication that is intended to treat PKU, including the use of large neutral amino acids, within 14 days prior to the administration of study drug on Day 1.

- Any injectable drugs containing polyethylene glycol (PEG; other than pegvaliase), including medroxyprogesterone injection, within 3 months prior to the start of Screening/Run-in and during study participation with the exception of COVID-19 vaccinations. Please contact the medical monitor prior to administering COVID-19 vaccinations.
- Use of any investigational product or investigational medical device within 30 days prior to Screening/Run-in or requirement for any investigational agent prior to completion of all scheduled study assessments.

In addition, if a participant is on medication for ADHD, depression, or other psychiatric disorder, the dose must be stable for ≥ 8 weeks prior to enrollment and must continue to be stable throughout the study unless a change is medically indicated.

6.8.2 Permitted and/or Required Concomitant Medications

All prescription and over-the-counter medication including herbal supplements and medical food taken by a participant for 30 days before screening are recorded on the designated CRF. Psychiatric medications and their dosage should remain consistent throughout the study unless a change is medically indicated. No new psychiatric medication should be initiated during the study unless medically indicated. No psychotherapy should be initiated during the study unless medically indicated. The investigator may prescribe additional medications during the study as long as the prescribed medication is not prohibited by the protocol (see Section 6.8.1). In the event of an emergency, any needed medications may be prescribed without prior approval but the medical monitor must be notified of the use of any contraindicated medication immediately thereafter. Any concomitant medication added or discontinued during the study should be recorded on the appropriate CRF.

All participants receiving pegvaliase will be required to premedicate with an H1 antagonist, and an H2 antagonist, and an antipyretic approximately 2 to 3 hours prior to each dose of study drug until they reach the Maintenance Phase, defined as when participants achieve blood Phe ≤600 µmol/L while they are on a stable dose of pegvaliase. Premedication may be considered during the Maintenance Phase at the investigator's discretion. For further information regarding premedication, refer to Section 6.5.

7 DISCONTINUATION OF STUDY TREATMENT AND PARTICIPANT WITHDRAWAL FROM STUDY

7.1 Discontinuation of Study Treatment

In rare instances it may be necessary for a patient to permanently discontinue study treatment. If a participant's blood Phe is > 600 µmol/L after 16 weeks of continuous treatment with pegvaliase at 60 mg/day, pegvaliase should be permanently discontinued.

If study treatment is permanently discontinued, the participant will be asked to continue to provide consent at the time of treatment discontinuation to remain in the study and undergo the scheduled study assessments off-treatment. Refer to the SoA for data to be collected at the time of discontinuation of study treatment and follow-up, and for any further evaluations that need to be completed.

If the participant declines to provide consent to remain in the study, the study completion visit assessments should be performed as detailed in the SoA (Section 1.3).

7.2 Participant Withdrawal from the Study

A participant may withdraw from the study at any time at his or her own request, or may be withdrawn at any time at the discretion of the investigator for safety, behavioral, compliance, or administrative reasons.

At the time of discontinuing from the study, if possible, an early termination (ET) visit should be conducted, as shown in the SoA (Section 1.3).

The participant will be permanently discontinued both from the study treatment and from the study at that time.

If the participant withdraws consent for disclosure of future information, the sponsor may retain and continue to use any data collected before such a withdrawal of consent.

If a participant withdraws from the study, BioMarin will only use the remaining samples to complete study procedures as described in Section 8 and no new samples will be collected. If a participant no longer wants the remaining samples to be used for future research, he/she shall inform the study investigator or institution accordingly and BioMarin will only use the remaining samples for health authority requests related to the activities in Section 8, not for future research. The investigator must document this in the site study records.

7.3 Lost to Follow-Up

A participant will be considered lost to follow-up if he or she repeatedly fails to return for scheduled visits and cannot be contacted by the site.

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

- The site must attempt to contact the participant to reschedule the missed visit as soon as possible, counsel the participant on the importance of maintaining the assigned visit schedule, and ascertain whether or not the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow up, the investigator or designee must make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record.
- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study.

Discontinuation of specific sites or of the study as a whole are handled as part of Appendix 1, Section 10.1.9.

8 STUDY ASSESSMENTS AND PROCEDURES

An ICF must be signed and dated by the participant's legally authorized representative, the investigator or designee, and a witness (if required) before any study-related procedures are performed.

All screening evaluations must be completed and reviewed to confirm that potential participants meet all eligibility criteria. The investigator will maintain a screening log to record details of all participants screened and to confirm eligibility or record reasons for screening failure, as applicable. Procedures conducted as part of the participant's routine clinical management (eg, blood count) and obtained before signing of the ICF may be utilized for screening or baseline purposes provided the procedures met the protocol-specified criteria and were performed within the time frame defined in the SoA.

Potential participants will enter the Screening/Run-in period, which will last 4 weeks and include 2 assessments of blood Phe concentration 7 to 10 days apart. Diet diaries should be completed for the 3 days preceding each of the blood Phe assessments. Following confirmation that the 2 blood Phe results are > 600 µmol/L and all other eligibility criteria have been met, participants may be enrolled in the study on Day 1. Protocol exemptions for eligibility are not allowed. Following enrollment, participants will be randomized 2:1 to the active (pegvaliase) or control (diet-only) treatment arm of the study.

In the Primary Treatment Phase (Part 1), participants in the active (pegvaliase) and control (diet-only) treatment arms will follow the same visit schedules and perform the same assessments except that the control participants will not be receiving pegvaliase so will not have PK draws or immunogenicity assessments. The timing of Part 1 assessments for active and control participants is provided in Table 1.3.1 and Table 1.3.4, respectively.

The primary efficacy endpoint, the change from baseline blood Phe, is assessed following 72 weeks on study (the end of Part 1). Beginning Week 73, participants in the active treatment arm will continue to receive pegvaliase in the Extension Phase (Part 2) for up to an additional 142 weeks (through Week 215). The timing of Part 2 assessments for active treatment participants is provided in Table 1.3.2 and Table 1.3.3.

Beginning Week 73, participants in the control treatment arm will initiate pegvaliase treatment and, from Week 73 through Week 215, will follow the same schedule for dosing, study visits, and assessments that the active arm participants followed. The timing of Part 2 assessments for control participants is provided in Table 1.3.5 and Table 1.3.6. All the assessments, dietary rules, and other restrictions that applied to active participants in the Primary Treatment Phase (Part 1) apply to control participants in Part 2 until the Week 145

visit when change from baseline blood Phe for these participants will be assessed after 73 weeks of pegvaliase treatment.

The last scheduled visit, which includes study drug administration, is at Week 215 for participants randomized to both the active (pegvaliase) and control (diet-only) treatment arms. Participants should perform the Study Completion (SC) Visit 4 weeks after the last dose of study drug or after completion of study participation, whichever occurs last. For participants who terminate from the study early, the Early Termination (ET) visit should occur within 4 weeks after the last study drug dose or after withdrawal from study participation, whichever occurs last. If study drug is discontinued before study completion, the investigator will ask the participant to remain in the study to continue study visits and assessments, provided the participant's health, safety, and welfare would not be detrimentally affected.

Immediate safety concerns should be discussed with the sponsor (medical monitor) immediately upon occurrence or awareness to determine if the participant should continue or discontinue study treatment.

Adherence to the study design requirements, including those specified in the SoA, is essential and required for study conduct.

8.1 Efficacy Assessments

Planned timepoints for all efficacy assessments are provided in the SoA (refer to Section 1.3).

8.1.1 Blood Phe Level

The primary efficacy endpoint is change in blood Phe concentration from treatment naïve baseline following 72 weeks on study (see Section 9.4.2). Blood samples for blood Phe analysis are to be drawn according to the SoA (see Section 1.3). Monitoring of blood Phe levels is the cornerstone of diagnosis and treatment of PKU patients. Guidelines for the treatment of PKU are based on blood Phe levels (Vockley 2014; van Wegberg 2017), and clinicians and patients use blood Phe concentration to assess and monitor PKU disease progression and control.

Participants must fast for at least 2.5 hours prior to blood Phe assessments to lessen the effect of food intake on blood Phe concentration. Samples for blood Phe concentration will be analyzed by a central laboratory.

8.1.2 Total Dietary Protein Intake, Dietary Monitoring, and Dietary Protocol Restrictions

Protein from medical foods (Phe-free amino acid fortified food sources) and protein from intact foods (any other food sources containing Phe) are collectively referred to as total dietary protein. Although total dietary protein intake is not an efficacy endpoint in this study, it is an important outcome measure that must remain stable for the primary endpoint to be accurately assessed and under the supervision of a dietician or adequately trained designee per the investigator during study participation. Specifically, to accurately assess the primary endpoint comparison between the active (pegvaliase) and control (diet-only) treatment arms on the change from baseline in blood Phe concentration following 72 weeks on study, total protein intake must remain stable for participants in both treatment arms during this period. It is essential that participants maintain stable protein intake to ensure that the measured changes in their blood Phe are attributable to study treatment rather than changes in their dietary protein intake. Total dietary protein is measured in grams/kilogram (g/kg).

A participant's diet is monitored using a 3-day diet diary. The 3-day dietary record (diet diary) is completed by participants, brought to clinic visits for review, and maintained with the study source documents. Participants record all food, beverages, special low-protein foods, and medical foods consumed for 3 consecutive days prior to the clinic visit and data are analyzed by the nutritional software (Metabolic Pro®) for total kcals, protein, Phe, tyrosine, and the percentage of daily recommended intake provided for protein, Phe, tyrosine, vitamins, and minerals.

A dietitian under investigator supervision is required to monitor and manage the participant's diet for the entire duration of the study, though an investigator or designee may also perform this role. Participants will be provided with 3-day diaries in which all dietary protein intake (including medical food and/or intact food) must be recorded for 3 consecutive days immediately prior to each scheduled clinic visit for review with the dietitian. The participant may be asked to repeat the 3-day diary collection if the dietitian or investigator/designee considers the diary has not been completed accurately; in such circumstances, study visits will be rescheduled. All participants will be provided the option of tyrosine supplementation (500 mg, 3 times per day with meals) at the discretion of the investigator.

The change from baseline in total protein intake at Week 73 will be compared as a secondary endpoint. For this endpoint, baseline protein intake is defined as the average of protein intake values derived from diet diaries collected during Screening/Run-in. During Screening/Run-in, 3-day diet diaries are completed 3 times, each time covering the 3 days periods prior to blood Phe assessments (and collected on the day of those assessments). The

3 Screening/Run-in blood Phe assessments are scheduled to occur (1) at Day -25 to Day -23; (2) 7 to 10 days later (ie, in the Day -18 to Day -13 window); and (3) at Day 1 pre-dose. Thus, the 3 day diet assessments will assess diet for (1) the 3 days within the Day -28 to Day -23 window immediately prior to the first blood Phe assessment; (2) the 3 days within the Day -21 to Day -15 window immediately prior to the second blood Phe assessment; and (3) for Days -3 to Day 1.

Participants will be instructed not to change their total dietary protein intake (medical food and/or intact food) during the Screening/Run-in and Primary Treatment Phase (Part 1) of the study. However, modifications to a participant's diet and reductions in study drug dose level must be implemented if blood Phe levels are reduced to < 30 µmol/L and confirmed upon recheck (performed within approximately 2 weeks) (see Section 8.1.2.1).

In Part 2, dietary protein intake may be modified for participants randomized to active (pegvaliase) treatment if blood Phe concentration is ≤ 360 µmol/L for a minimum of 4 weeks. Should that occur, participants may adjust dietary protein intake based on individual participant response to pegvaliase and guidance from the investigator or designee (see Section 8.1.2.2). Participants randomized to the control (diet-only) treatment arm must continue to maintain dietary protein intake consistent with baseline through Week 145 except in the case of blood Phe levels < 30 µmol/L confirmed upon recheck (see Section 8.1.2.1).

Baseline total dietary protein intake will be used as the comparator for subsequent 3-day diet diary entries. Participants will be required to maintain dietary protein intake levels that are consistent with their baseline levels for the entire duration of the study, except as otherwise specified above, with a consistent diet defined as one in which the intact protein changes are < 15% from baseline and the medical food protein changes are < 15% from baseline.

If a participant demonstrates ≥ 15% change from baseline intake of total intact protein and/or medical food protein, the dietitian (or investigator/designee) will counsel the participant to resume their baseline diet and may perform another 3-day diet record and blood Phe concentration assessment in 2 weeks. If the participant demonstrates ≥ 15% increase in intake of protein from intact foods and/or medical food at the next scheduled study visit, a discussion with the medical monitor may be required to discuss further actions related to non-adherence with consistent dietary protein intake throughout the study, at the discretion of the investigator.

If dietary protein intake is determined to be inconsistent with baseline values, the study site dietitian (or investigator/designee) should counsel the participant on how to return to baseline values by modifying intake of protein from intact foods and/or medical food. If diet changes have been implemented, a blood Phe concentration assessment and 3-day diet record should

be collected 2 weeks after implementation, in addition to all regularly scheduled assessments. Any changes must also be documented on the appropriate eCRF.

8.1.2.1 Diet Modification for Blood Phe Levels Less than 30 µmol/L

In addition to protocol required dose reductions (Section 6.5), modifications to participant's diet should be performed if blood Phe levels are < 30 µmol/L and confirmed upon repeat blood Phe measurement (performed within approximately 2 weeks). Participants will receive counseling for modification of intake of intact protein and medical foods based on intake at the time of blood Phe assessment, as follows:

- If it is determined that a participant is consuming < 2x their recommended level of intact protein per Dietary Reference Intake (DRI) guidelines (1.6 g/kg/day), the dietitian may instruct the participant to increase their intact protein by 20 grams/day and decrease their medical food protein by 20 grams/day.
- If it is determined that a participant is consuming ≥ 2x their recommended level of intact protein per DRI guidelines, the dietitian may instruct the participant to increase their intact protein by 10 grams/day and decrease their medical food protein by 10 grams/day.

Further increases in protein intake as per above are allowed if blood Phe levels are < 30 µmol/L in order to achieve 2 x DRI guidelines (1.6 g/kg/day) of intact protein intake. This increased intake will now be the new reference baseline for dietary protein intake for future dietary assessments. Medical food may be discontinued once the dietitian determines that the essential amino acids meet the appropriate levels for age.

8.1.2.2 Dietary Changes for Blood Phe ≤360 µmol/L in Extension Phase (Participants Randomized to Pegvaliase)

Once participants initially randomized to active (pegvaliase) treatment enter the Extension Phase (Part 2) of the study, dietary protein intake may be modified if blood Phe concentration reaches ≤ 360 µmol/L for a minimum of 4 weeks. If diet is modified, it is recommended to increase protein from intact foods in 10 or 20 gram increments depending on intact protein intake, and decrease protein from medical food by a similar amount, 10 or 20 grams unless the participant is already consuming the appropriate protein levels for age (World Health Organization 2007). Medical food may be discontinued once the dietitian or investigator/designee determines that the essential amino acids provided from intact protein foods meet the appropriate levels for age.

From the beginning of the Extension Phase (Part 2), participants initially randomized to active (pegvaliase) treatment who have achieved blood Phe > 30 to ≤360 µmol/L may have their medical food intake reduced or discontinued and intact protein intake increased, in

accordance with Table 8.1.2.2.1. Implementation of dietary adjustments will be performed in consultation with the site dietitian.

Table 8.1.2.2.1: Dietary Adjustment Algorithm in Part 2 for Participants Randomized to Active (Pegvaliase) Treatment

Phe Category [a]	Protein Intake from Intact Food Sources [b]	Action	
		Protein Intake from Intact Food Sources	Protein Intake from Medical Food
> 30 [d] to ≤ 360 µmol/L	> 2 × RDI (> 1.7 g/kg)	Maintain or reduce by 10 g/day [c]	Discontinue [c]
	> 1 × to 2 × RDI (> 0.8 to 1.7 g/kg)	Maintain	Discontinue [c]
	0.5 × to 1 × RDI (0.4 to 0.8 g/kg)	Increase 10 g/day [c]	Decrease 10 g/day [c]
	< 0.5 × RDI (< 0.4 g/kg)	Increase 20 g/day [c]	Decrease 20 g/day [c]

RDI, recommended daily intake.

[a] Based on results of the previous blood Phe assessment; blood Phe reductions may not be observed until the participant is titrated to an effective dose.

[b] RDI (0.83 g/kg) is based on the joint FAO/WHO/UNU Expert Consultation on Protein and Amino Acid Requirements in Human Nutrition (World Health Organization 2007).

[c] The resulting adjusted dietary protein intake will become the new reference baseline for dietary protein intake for future dietary assessments.

[d] If a participant experiences blood Phe levels < 30 µmol/L on two consecutive blood tests, refer to the action to take in the event of blood Phe ≤ 30 µmol/L (Section 8.1.2.1).

8.1.3 Exploratory Assessments

8.1.3.1 Neurocognitive Assessments

The ADHD RS-IV and BRIEF2 instruments will be used to evaluate the effect of pegvaliase treatment on neurocognitive outcomes in adolescent participants with PKU. The same parent/guardian must complete both the ADHD RS-IV and the BRIEF2 at all scheduled time points.

8.1.3.1.1 The ADHD Rating Scale IV (ADHD RS-IV) Inattention Subscale

The ADHD RS-IV (American Psychiatric Association 2013; DuPaul 1998) will be used to assess inattention and hyperactivity symptoms among the adolescents enrolled in this study. The ADHD RS-IV is derived directly from diagnostic criteria delineated in the DSM, is well validated, and has been extensively used in a broad array of disease classes, including PKU (Gassio 2010; Wyrwich 2016). The ADHD RS-IV was the secondary endpoint in the

BioMarin Phase 3 pegvaliase study (165-302) in which improvement in inattention symptoms was associated with reduction in mean blood Phe levels (Thomas 2018).

The ADHD RS-IV instrument is completed independently by the parent/guardian. The same parent/guardian must complete the scale at each scheduled time point. The instrument consists of 2 nine-item scales that evaluate symptom severity for inattention and hyperactivity/impulsivity, respectively, during the previous month. Each scale is scored separately. Each item is rated on a 4-point severity scale (0, none; 1, mild; 2, moderate; 3, severe); scores of 2 or 3 on an individual item indicate a symptom. The PKU literature suggests that inattentive symptoms are more prevalent than hyperactivity and impulsivity symptoms among individuals with PKU; thus, the inattentive sub-scale is considered the most relevant and will be included in analyses (Bilder 2016).

8.1.3.1.2 Behavior Rating Inventory of Executive Function, Second Edition (BRIEF2)

The BRIEF2 is a 63-item measure designed to assess executive function skills in children 5 to 18 years of age (Gioia 2002). The BRIEF2 was developed to address the limitations of available assessments in examining real-word expressions of behaviors related to executive function. The BRIEF2 is intended to provide a standard method of asking multiple raters about executive function in daily life in a manner that is not specific to any particular disease. Because it is non-disorder-specific, BRIEF2 assesses executive function behaviors in children and adolescents experiencing a wide range of difficulties, such as those related to learning, attention, brain injuries, development disorders, and various psychiatric conditions and medical issues. The BRIEF2 is most often used to assess attention deficit hyperactivity disorder.

The BRIEF2 instrument (completed by the parent/guardian) contains 63 items in 8 non-overlapping clinical scales and 2 validity scales. The same parent/guardian must complete the BRIEF2 at each scheduled time point. These scales form 2 indexes: (1) Behavior Regulation (3 scales) and (2) Metacognition (5 scales), as well as a Global Executive Composite score which takes into account all of the clinical scales and represents the adolescent's overall executive function.

8.1.3.1.3 PKU Symptom Severity and Impacts Scale (PKU-SSIS) v2.0 – Adolescent Version

The PKU Symptom Severity and Impact Scale (PKU-SSIS) was designed to evaluate neuropsychological symptoms and impacts in early-treated patients with PKU. The PKU-SSIS is comprised of 22 items that constitute 3 symptoms domains: 1) Emotional, Mood and Psychological; 2) (Neuro)cognitive, Executive, and Intellectual Function; 3) Physical Health.

It also has 4 impact domains: 1) Social Relations; 2) Level of Independence; 3) General Wellbeing; and 4) Self-care (Quinn 2022). PKU-SSIS Version 2.0 is developed specifically for adolescents 12 to 17 years old. The participant completes the PKU-SSIS v2.0 independently. Each item is scored on a 5-point Likert scale which asks participants to report how frequently they have experienced symptoms over the last 7 days between "Never," "Rarely," "Occasionally," "Frequently," and "Very Frequently." For each point of the Likert scale the following numerical value is given: Never=0, Rarely=1, Occasionally=2, Frequently=3, and Very Frequently=4. Two items on the scale ask the participants to rate their satisfaction with social interactions and leisure time over the last 7 days; these items are scored on a 5-point Likert scale ranging from "Very Dissatisfied" to "Very Satisfied." For each point of the Likert scale the following numerical value is given: Very Dissatisfied=0, Dissatisfied=1, Neutral=2, Satisfied=3, and Very Satisfied=4.

Scores to items 14, 17, 21, and 22 are reversed so that lower scores represent less symptom severity. Each domain score is computed by summing all the item scores within each domain and converting to a percentage score from the highest possible score from that domain. The instrument total score is compiled by summing the domain scores and converting to a percentage score (ie, Total score = (summed score/88)*100). Participants will complete the PKU-SSIS as indicated in the SoA.

8.1.3.2 Global Impression Scales

8.1.3.2.1 Caregiver Global Impression of Severity (CaGIS) and Caregiver Global Impression of Change (CaGIC)

The Caregiver Global Impression of Severity (CaGIS) and Change (CaGIC) items are standard, single items that have been widely used across a broad range of disease areas and are typically included in clinical trials as anchor measures to help establish meaningful change in other COAs. The CaGIS is used to rate the overall disease severity, as reported by the parent/guardian, and includes a 5-point Likert scale from "None/Not at all impacted" to "Very severe/Very severe impact." The CaGIC is a single global item used to assess if there has been an improvement or change in clinical status; the item includes a 5-point Likert scale from "Much better" to "Much worse." Both global and domain specific items will be created for the CaGIS and CaGIC. Parents/guardians will complete the CaGIS and CaGIC at baseline (CaGIS only) and as indicated in the SoA.

8.1.3.2.2 Patient Global Impression of Severity (PGIS) and Patient Global Impression of Change (PGIC)

The Patient Global Impression of Severity (PGIS) and Change (PGIC) items are standard, single items that have been widely used across a broad range of disease areas and are typically included in clinical trials as anchor measures to help establish meaningful change in other COAs. The PGIS is used to rate the overall disease severity, as reported by the participant, and includes a 5-point Likert scale from "None/Not at all impacted" to "Very severe/Very severe impact." The PGIC is a single global item used to assess if there has been an improvement or change in clinical status; the item includes a 5-point Likert scale from "Much better" to "Much worse." Both global and domain specific items will be created for the PGIS and PGIC. Participants will complete the PGIS and PGIC at baseline (PGIS only) and as indicated in the SoA.

8.1.3.2.3 Clinician Global Impression of Severity (CGIS)

The Clinician Global Impression of Severity (CGIS) is a standard, single global item that has been widely used across a broad range of disease areas and are typically included in clinical trials as anchor measures to help establish meaningful change in other COAs. The CGIS is used to rate the overall disease severity, as reported by the investigator or designee clinician, and includes a 5-point Likert scale from "None" to "Very severe." An overall severity item will be created for the CGIS. The investigator or designee clinician will complete the CGIS at baseline and as indicated in the SoA.

8.2 Safety Assessments

Planned time points for all safety assessments are provided in the SoA.

8.2.1 Physical Examination

Physical examinations (full or brief) should be performed at the timepoints indicated in the SoA:

- A full physical examination will include, at a minimum, general appearance (head, eyes, ears, nose, and throat) and an assessment of cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, musculoskeletal, and neurologic systems.
- A brief physical examination may be performed at the discretion of the investigator based on the participant's clinical condition and may include, but is not limited to, general appearance and an assessment of cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, musculoskeletal, and neurologic systems.

Investigators should pay special attention to clinical signs related to previous serious illnesses.

Height and weight will also be measured and recorded at the timepoints indicated in the SoA (Section 1.3).

8.2.2 Vital Signs

Vital signs include respiration rate, temperature, and single measurements of heart rate and seated diastolic and diastolic blood pressure. Any clinically significant change in vital signs will be recorded as an AE. Blood pressure and pulse measurements should be preceded by at least 5 minutes of rest for the participant in a quiet setting without distractions (eg, television, cell phones). Blood pressure and pulse measurements will be assessed with a completely automated device. Manual techniques will be used only if an automated device is not available.

Results will be recorded on the eCRF. Any abnormal vital sign assessments should be repeated once, and repeat value should be recorded in the eCRF.

8.2.3 Electrocardiograms

Electrocardiograms (ECGs) will be performed at Screening, Baseline, and SC/ET, as shown in the SoA (Section 1.3). Single 12-lead ECG will be obtained using an ECG machine that automatically calculates the heart rate and measures PR, QRS, QT, and QTc intervals.

8.2.4 Clinical Safety Laboratory Assessments

See Appendix 2, Section 10.2 for the table of clinical laboratory tests to be performed and refer to the SoA (Section 1.3) for the timing and frequency.

The investigator must review the laboratory report and document this review. The laboratory reports must be filed with the source documents. If known, the underlying diagnosis associated with abnormal clinical laboratory test results that are considered clinically significant by the investigator should be recorded on the AE eCRF. Clinically significant abnormal laboratory findings are those which are not associated with the underlying disease, unless judged by the investigator to be more severe than expected for the participant's condition.

All abnormal laboratory tests with values considered clinically significant per the investigator during participation in the study and 30 days after the last dose of study treatment should be repeated until the values return to normal or baseline or are no longer considered clinically significant by the investigator.

In the event of elevated urinary protein on a test result, a repeat urinalysis should be performed. This repeat urine sample must be performed in the morning at the first or second morning void to allow for accurate test results and may be performed by a home healthcare nurse. Participants with a confirmed urine/albumin creatinine ratio of ≥ 100 mg/g should be

referred to a nephrologist for consultation if results were within normal range at Baseline. Participants who had elevated results at Baseline followed by a confirmed subsequent increase of ≥ 100 mg/g from Baseline should also be referred to a nephrologist for consultation.

If clinically significant values do not return to normal/baseline within a period of time judged reasonable by the investigator, the etiology should be identified and the medical monitor notified.

All protocol-required laboratory assessments, as defined in Appendix 2, Section 10.2, must be conducted in accordance with the laboratory manual and the SoA.

If laboratory values from non-protocol specified laboratory assessments performed at the institution's local laboratory require a change in participant management or are considered clinically significant by the investigator (eg, SAE or AE or dose modification), then the results must be recorded in the eCRF.

8.2.5 Pregnancy Testing

Based on findings in studies of pregnant animals without PKU treated with pegvaliase-pqpz, pegvaliase may cause fetal harm when administered to a pregnant woman. Because the risks of taking study drug during pregnancy and breastfeeding in humans are unknown, participants must not take study drug if they are trying to conceive, are pregnant, or are breastfeeding. From Day –28 until the end of the study, sexually active participants must use 2 acceptable methods of contraception as outlined in the inclusion criteria section of this protocol (Section 5.1). Participants who are confirmed to be pregnant by a serum pregnancy test and are temporarily off study drug are not required to perform the scheduled urine pregnancy tests.

In addition to contraception requirements, females of child-bearing potential are required to have urine pregnancy tests performed at the timepoints specified in the SoA (Section 1.3). Urine pregnancy test should be performed by the local laboratory. A serum pregnancy test will be performed by the central laboratory in the event of a positive or equivocal urine pregnancy test result.

Refer to Section 8.3.5 for details on the reporting procedures in the event of pregnancy.

8.3 Adverse Events and Serious Adverse Events

The definitions of an AE or SAE can be found in Appendix 3, Section 10.3.

AEs will be reported by the participant (or, when appropriate, by a caregiver, surrogate, or the participant's legally authorized representative).

The investigator and any medically qualified designees are responsible for identifying, documenting, and recording events that meet the definition of an AE or SAE and remain responsible for following up AEs that are serious, considered related to the study treatment or study procedures, or that caused the participant to discontinue the study treatment (refer to Section 7).

8.3.1 Time Period and Frequency for Collecting AE and SAE Information

Control (diet-only) treatment arm participant reporting:

- After informed consent to Day 1, only SAEs related to protocol mandated procedures will be collected.
- All AEs, SAEs, AESI, pregnancy and device related events will be collected from Day 1 through 4 weeks after last dose of study treatment or the study completion visit, whichever is longer.
- Non serious events that occur after informed consent but prior to Day 1 will be recorded on the Medical History/Concurrent Medical Conditions eCRF, not the AE eCRF.

Active (pegvaliase) treatment arm participant reporting:

- After informed consent through the first dose of study treatment, only SAEs related to protocol mandated procedures will be collected.
- All AEs, SAEs, AESI, pregnancy, and device-related events will be collected after the first dose of study treatment through 4 weeks after the last dose of study treatment or the study completion visit, whichever is longer. Non serious events that begin after informed consent but before study treatment will be recorded on the Medical History/Concurrent Medical Conditions eCRF, not the AE eCRF.

All SAEs and AESIs must be recorded and reported to the sponsor or designee immediately; under no circumstances should this exceed 24 hours after the site becomes aware of the event, as indicated in Appendix 3, Section 10.3. The investigator must submit any updated SAE data to the sponsor within 24 hours of it being available.

Investigators are not obligated to actively seek AE or SAE information after conclusion of study participation. However, if the investigator learns of any SAE, including a death, at any time after a participant has been discharged from the study and he/she considers the event to be reasonably likely to be related to the study treatment or study participation, the investigator must promptly notify the sponsor.

8.3.2 Method of Identifying AEs and SAEs

The method of recording, evaluating, and assessing causality of AEs and SAEs and the procedures for completing and transmitting SAE reports are provided in Appendix 3, Section 10.3.

Care will be taken not to introduce bias when identifying AEs and/or SAEs. Open-ended and non-leading verbal questioning of the participant is the preferred method to inquire about AE occurrences.

8.3.3 Follow-up of AEs and SAEs

After the initial AE/SAE report, the investigator is required to proactively follow each participant at subsequent visits/contacts. All SAEs and AESIs (as defined in Section 8.3.8) will be followed until resolution, stabilization, the event is otherwise explained (ie, not related to study drug and/or study participation), or the participant is lost to follow-up (as defined in Section 7.3). Further information on follow-up procedures is provided in Appendix 3, Section 10.3.

8.3.4 Regulatory Reporting Requirements for SAEs

Prompt notification by the investigator to the sponsor of a SAE is essential so that legal obligations and ethical responsibilities towards the safety of participants and the safety of a study treatment under clinical investigation can be met.

The sponsor has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a study treatment under clinical investigation. The sponsor will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority, Institutional Review Boards (IRB)/Independent Ethics Committees (IEC), and investigators.

Expedited safety reports must be prepared for suspected unexpected serious adverse reactions according to global regulatory requirements and sponsor policy and forwarded to investigators.

An investigator who receives an expedited safety report or other specific safety information from the sponsor will review and then file it along with the IB and will notify the IRB/IEC as appropriate.

8.3.5 Pregnancy

The importance of pregnancy prevention for study participants and their partners will be communicated to all study participants with regular reminders during the study.

Any female participant who becomes pregnant while participating in the study will temporarily discontinue study treatment.

Details of all pregnancies in female participants and, if indicated, female partners of male participants, will be collected after the start of study treatment and until 4 weeks after the last dose of study treatment.

While pregnancy itself is not considered to be an AE or an SAE, any pregnancy complication or elective termination of a pregnancy for medical reasons will be reported as an AE or SAE.

Abnormal pregnancy outcomes (eg, spontaneous abortion, fetal death, stillbirth, congenital anomalies, ectopic pregnancy), as well as any hospitalizations associated with pregnancy complications, unplanned caesarean section, and delivery complications should always be classified as an SAE (as the sponsor considers these to be medically significant), recorded on the eCRF, and expeditiously reported to the sponsor as an SAE following the procedures outlined in Appendix 3, Section 10.3.4.

The participant (or pregnant female partner) will be followed to determine the outcome of the pregnancy. The investigator will collect follow-up information on the participant (or pregnant female partner) and the neonate, and the information will be forwarded to the sponsor.

Any post-study pregnancy-related SAE considered reasonably related to the study treatment by the investigator should be reported to the sponsor as described in Section 8.3.4. While the investigator is not obligated to actively seek this information in former study participants (or their pregnant female partners), he or she may learn of an SAE through spontaneous reporting.

Pregnancy reporting requirements to BioMarin are shown in Table 8.3.5.1.

Table 8.3.5.1: Pregnancy Reporting to BioMarin

Pregnancy Reporting to BioMarin
• Pregnancy and Pregnancy Outcome information will be entered into the electronic data collection system. • Pregnancy and Pregnancy Outcome information will be reported to BPV via the study specific Pregnancy Report and Pregnancy Outcome paper report forms. The site will complete the forms and submit via facsimile or email without 24 hours of awareness. • Abortion, Stillbirth, Fetal Congenital Anomaly and any other event meeting seriousness criteria must be reported as an SAE via the study electronic data collection system.

Male Participants with Partners Who Become Pregnant

The investigator will attempt to collect pregnancy information on any male participant's female partner who becomes pregnant while the male participant is in this study. This applies only to male participants who receive pegvaliase.

After obtaining the necessary signed informed consent from the pregnant female partner directly, the investigator will record pregnancy information on the appropriate form and submit it to the sponsor within 24 hours of learning of the partner's pregnancy. The female partner will also be followed to determine the outcome of the pregnancy. Information on the status of the mother and child will be forwarded to the sponsor. Generally, the follow-up will be no longer than 6 to 8 weeks following the estimated delivery date. Any termination of the pregnancy will be reported regardless of fetal status (presence or absence of anomalies) or indication for the procedure.

8.3.6 Cardiovascular and Death Events

All deaths that occur during the AE reporting period, regardless of attribution, will be recorded on the AE eCRF and expeditiously reported to the Sponsor as an SAE.

When recording a death, the event or condition that caused or contributed to the fatal outcome should be recorded as the single medical concept on the eCRF. If the cause of death is unknown and cannot be ascertained at the time of reporting, record "Unexplained Death" or "Death of Unknown Cause" on the eCRF.

8.3.7 Disease-Related Events and/or Disease-Related Outcomes Not Qualifying as AEs or SAEs

The following disease-related event (DRE) is common in participants with PKU and can be serious:

- Hyperphenylalaninemia (Medical Dictionary for Regulatory Activities terminology [MedDRA] term: amino acid increased)

Because this event is typically associated with the disease under study, it will not be reported according to the standard process for expedited reporting of SAEs even though the event may meet the definition of a SAE. The elevated blood Phe levels should be recorded in the central laboratory database as per standard practice. These DREs will be monitored on a routine basis.

If either of the following conditions applies, then the event must be recorded and reported as an SAE (instead of a DRE):

- The event is, in the investigator's opinion, of greater intensity, frequency, or duration than expected for the individual participant; OR
- The investigator considers that there is a reasonable possibility that the event was related to study treatment.

8.3.8 Adverse Events of Special Interest

The following adverse events of special interest (AESI) must be reported to BPV within 24 hours of site awareness, irrespective of seriousness, severity, or causality to facilitate real-time monitoring and assessment. All AESI must be reported using the SAE form regardless of seriousness:

- Acute systemic hypersensitivity reaction, anaphylactic, or anaphylactoid reactions (refer to Appendix 5, Section 10.5)
- Angioedema
- Serum sickness
- Severe injection site reaction defined as an injection site reaction that is considered CTCAE Grade ≥ 3
- Hypophenylalaninemia events (defined as blood Phe < 30 µmol/L on 2 consecutive measurements)
- Arthralgia that is either persistent (AE duration > 6 months), severe (CTCAE Grade ≥ 3), or that requires treatment with a steroid (excluding topical)

8.3.8.1 Anaphylaxis (Acute Systemic Hypersensitivity Reaction)

All potential anaphylactic events, however reported, will be reviewed by BioMarin. When an event occurs which an investigator believes is possibly anaphylaxis, in addition to reporting the event to BPV within 24 hours of site awareness, it should be reported to the sponsor's medical monitor within 24 hours of site awareness.

In this protocol the term anaphylaxis is used to denote the important identified risk: acute systemic hypersensitivity reaction (see Section 2.3.1). An acute systemic hypersensitivity reaction (referred to as anaphylaxis in this protocol) is defined as an episode meeting National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network (NIAID/FAAN) criteria according to an internal BioMarin assessment performed by the Internal Data Safety Review Board. The Internal Safety Data Review Board, consisting of a representative from BioMarin clinical sciences, safety, and biostatistics departments, will review all cases of potential anaphylaxis to determine which of these events meet the criteria for an acute systemic hypersensitivity reaction as summarized in Appendix 5, Section 10.5.2.

In the clinical study report, analyses of anaphylaxis will include both the definition used in this protocol of an acute systemic hypersensitivity reaction and the FDA's definition of anaphylaxis, developed by the Division of Pulmonary, Allergy, and Rheumatology Products (DPARP) and used by BioMarin in the Palynziq US prescribing information (see the SAP for further details).

8.3.8.1.1 Individual Stopping Criteria Evaluation for Adverse Events During Pegvaliase Treatment

A participant who has an anaphylaxis episode that is suspected to meet Brown's criteria for severe (Grade 3) and that is, in the judgment of the investigator and/or the sponsor's medical monitor, related to study drug, will be permanently discontinued from study drug. Clinically severe hypersensitivity (Grade 3) per Brown's criteria is defined as significant hypoxia, hypotension or neurologic compromise that is life-threatening or required treatment to prevent a life-threatening event (Brown 2004):

- Cyanosis or $SpO_2 \leq 92\%$
- Hypotension with SBP < 90 mmHg
- Neurologic alteration: confusion, loss of consciousness, collapse, incontinence

In addition, a participant with a recurrent anaphylaxis episode of any severity that is, in the judgment of the investigator and/or the sponsor's medical monitor, related to study drug will be permanently discontinued from treatment.

8.3.8.1.2 Response to Anaphylaxis Episodes

If a participant experiences any symptoms suggestive of an anaphylaxis episode (as taught to the participant and adult observer in the initial and ongoing training on this risk), they will be instructed to inject epinephrine, to seek immediate medical attention, *and* to inform the investigator.

If the investigator suspects that an AE is anaphylaxis as defined by NIAID/FAAN, the participant will be assessed in the clinic and the sponsor's medical monitor must be immediately notified. Laboratory assessments for suspected anaphylaxis events described in the SoA (Section 1.3) under Hypersensitivity Reaction Visit (HRV) and in Table 6.5.1.1 should be performed prior to the next administration of study drug (if applicable) and should include anti-pegvaliase IgE (for optimal results, sampling should be performed 8 to 24 hours after event onset). If the investigator determines it is safe for the participant to resume dosing with study drug following resolution of anaphylaxis at any time during the study (excepting episodes requiring participant discontinuation, as outlined above), the following steps are required:

- At least the first dose administered after resolution of anaphylaxis is to be given at the clinic with equipment for emergency resuscitation (including epinephrine) within easy access.
- The participant must be premedicated with an H1 antagonist, and an H2 antagonist, and an antipyretic (eg, acetaminophen) approximately 2 to 3 hours prior to each dose of study drug for at least 1 week upon resumption of dosing, regardless of the duration of dose interruption.
- As with all doses of pegvaliase, an adult observer must observe the participant during study drug administration and for a minimum of 1 hour following study drug administration.

8.3.8.2 Angioedema

Angioedema is an eruption similar to urticaria but with larger, edematous wheals involving dermal, subcutaneous or submucosal tissues. It is sometimes associated with severe respiratory distress due to edema of the upper airways. Angioedema may present as a symptom (eg, lip, eye, tongue, throat, or facial swelling) of an acute systemic hypersensitivity reaction or anaphylaxis but may also present in isolation.

8.3.8.3 Serum Sickness

Serum sickness is a Type III immune hypersensitivity reaction that occurs when the immune system develops antibodies to a foreign protein. The antibody and foreign protein combine to form an immune complex that can settle in areas of the body such as the joints or the kidney. Typical presentations include signs and symptoms commonly associated with hypersensitivity or infections: rash, itching, fever and arthralgia (MedDRA terms: serum sickness, serum sickness-like reaction and Type III immune-complex mediated reaction).

8.3.8.4 Severe Injection Site Reaction

A severe injection site reaction will be defined as any injection site reaction adverse event that is reported as CTCAE Grade 3 or above in severity and that codes to the MedDRA high level term (HLT) of Injection site reaction.

8.3.8.5 Hypophenylalaninemia

Blood Phe levels will be regularly monitored during the study. Hypophenylalaninemia is defined in this study as 2 consecutive blood Phe levels < 30 µmol/L. Hypophenylalaninemia will be managed in this study as described in Section 6.5.

8.3.8.6 Arthralgia

Arthralgia will be identified using the MedDRA PT of arthralgia and will include any synonyms of arthralgia such as musculoskeletal pain, pain in the extremity, or neck pain.

Any event that meets these criteria and has a duration of > 6 months will be considered a persistent arthralgia. An arthralgia event that is reported as CTCAE Grade 3 or above in severity will be considered a severe event. Arthralgia events that require treatment with a steroid medication will also be considered an AESI.

8.3.9 Medical Device Incidents (Including Malfunctions)

Medical devices (prefilled syringes) are being provided for use in this study. In order to fulfill regulatory reporting obligations, the investigators are responsible for the detection and documentation of events meeting the definitions of device deficiency or malfunction that occur during the study with such devices.

The definition of a Medical Device deficiency can be found in Appendix 6, Section 10.6.

NOTE: Incidents fulfilling the definition of an AE/SAE will also follow the processes outlined in Section 8.3.3 and Appendix 3, Section 10.3.

8.3.9.1 Time Period for Detecting Medical Device Deficiencies

Medical device incidents or malfunctions of the device that result in an incident will be detected, documented, and reported during all periods of the study in which the medical device is used.

If the investigator learns of any device deficiency at any time after a participant has been discharged from the study, and such incident is considered reasonably related to a medical device provided for the study, the investigator will promptly notify the sponsor.

The method of documenting Medical Device deficiencies is provided in Appendix 6, Section 10.6.

8.3.9.2 Follow-up of Medical Device Deficiencies

Follow-up applies to all participants, including those who discontinue study treatment.

The investigator is responsible for ensuring that follow-up includes any supplemental investigations as indicated to elucidate the nature and/or causality of the deficiency.

New or updated information will be recorded on the originally completed form with all changes signed and dated by the investigator.

8.3.9.3 Prompt Reporting of Device Deficiencies to Sponsor

Device deficiencies must be reported to the sponsor within 24 hours after the investigator determines that the event meets the protocol definition of a medical device deficiency.

The investigator should complete the Device Malfunction eCRF in electronic data capture (EDC). The investigator/designee must complete a Product Complaint form and submit it to the sponsor via fax or email as outlined in the Pharmacy Manual. The sponsor will be the contact for the receipt of device deficiency reports.

8.3.9.4 Regulatory Reporting Requirements for Device Deficiencies

The investigator must promptly report all device deficiencies occurring with any medical device provided for use in the study so the sponsor can fulfill their legal responsibility to notify appropriate regulatory authorities and other entities about certain safety information relating to medical devices being used in clinical studies.

The investigator, or responsible person according to local requirements (eg, the head of the medical institution), will comply with the applicable local regulatory requirements relating to the reporting of device deficiencies to the IRB/IEC.

8.4 Pharmacokinetics

Blood samples will be collected for measurement of plasma concentrations of pegvaliase as specified in the SoA.

For participants randomized to active (pegvaliase) treatment, Part 1 blood samples for trough PK will be collected pre-dose (within 2 hours of dosing) at Day 1 and every 4 weeks for the first 24 weeks, followed by sampling every 8 weeks thereafter during Part 1. Intensive PK sampling will be performed at Week 73 and at dose escalations to 20, 40, and 60 mg/day. For intensive PK, samples will be taken at pre-dose (within 2 hours of dosing); at 2, 4, 8, and 12 hours (± 30 minutes) post-dose; and at 24 hours (± 2 hours) post dose. The 24-hour sample will be taken prior to the next daily dose. Extension Phase (Part 2) trough PK samples will be collected every 8 weeks.

For participants randomized to control (diet-only) treatment, there are no PK assessments in Part 1. In Part 2, the PK assessment schedule will mirror the Part 1 schedule performed for active participants, with trough PK samples collected pre-dose (within 2 hours of dosing) at Week 73 and every 4 weeks for the first 24 weeks and then every 8 weeks though the end of the study. Intensive PK sampling will be performed at Week 145 and for dose escalations to 20, 40, and 60 mg/day. For intensive PK, samples will be taken at pre-dose (within 2 hours of dosing); at 2, 4, 8, and 12 hours (± 30 minutes) post-dose; and at 24 hours (± 2 hours) post dose.

The PK parameters shown in Table 8.4.1 will be determined if feasible from the plasma concentration-time data for participants who have sufficient data for PK analysis (eg, at least 3 samples with measurable concentrations during the terminal log-linear phase for estimating

the terminal half-life and related parameters; at least 3 samples with measurable concentrations for other derived parameters).

Table 8.4.1: Derived Parameters for Pharmacokinetics Analysis

Parameter	Parameter Description
$AUC_{0-24hr,ss}$	Area under the plasma concentration-time curve from 0 to 24 hours at steady state, calculated using the linear trapezoidal rule
$C_{max,ss}$	Maximum observed plasma concentration within 24 hours post-dose at steady state
$T_{max,ss}$	Time to reach Cmax,ss
$C_{trough,ss}$	Trough observed plasma concentration prior to dosing at steady state
$t_{1/2}$	Terminal half-life, t1/2=ln2/λz where λz is the elimination rate constant
$CL_{ss/F}$	Apparent clearance of drug following subcutaneous injection at steady state
$V_{dz/F}$	Apparent volume of distribution based upon the terminal phase

The timing of sampling may be altered during the course of the study based on newly available data (eg, to obtain data closer to the time of peak plasma concentrations) to ensure appropriate monitoring. Instructions for the collection and handling of biological samples will be provided by the sponsor. The actual date and time (24-hour clock time) of each sample will be recorded.

Plasma samples collected for analyses of pegvaliase concentration may also be used for exploratory safety or efficacy analyses during or after the study.

Genetic analyses will not be performed on these plasma samples.

8.5 Pharmacodynamics

Blood Phe and Tyrosine (Tyr) will be measured by validated assays (see Section 8.1.1 and Section 1.3).

8.6 Biomarkers and Genetic Assessment

8.6.1 PAH Genotyping

PAH genotype testing should be performed where allowed by local health authorities and the EC/IRB.

8.6.2 Blood for Exploratory Analyses

As an exploratory analysis, blood will be collected to evaluate biochemical, molecular, and cellular aspects of PKU and to develop the assays used for these evaluations. The blood samples reserved for exploratory analyses are collected at the time points indicated in the SoA (see Section 1.3).

For each portion of blood samples reserved for protocol-specified analyses, there may be multiple sample aliquots. Once samples have been successfully analyzed during the study per protocol, unused sample portions may be used during the study and afterwards for assay development and other purposes stated in this section.

8.7 Immunogenicity Assessments

Sampling for immunogenicity testing will be performed routinely throughout the study to provide a comprehensive assessment of the immune response against pegvaliase. Antibodies against pegvaliase will be evaluated in serum samples collected from all participants according to the SoA (Section 1.3). Additionally, serum samples should be collected at the final visit from participants who discontinued study treatment or were withdrawn from the study.

Routine immunogenicity testing for anti-drug antibodies will be performed using validated semi-quantitative assays used in previous Phase 3 studies. Titer and incidence of positivity for each of the following analytes will be reported: total anti-pegvaliase antibodies (TAb), anti-PAL IgG, anti-PAL IgM, anti-PEG IgM, anti-PEG IgG, and pegvaliase-neutralizing antibodies (NAb). In the event of a hypersensitivity reaction visit (HRV) only, assessment of anti-pegvaliase IgE antibodies will be performed using an ImmunoCAP assay after depletion of IgG and IgM.

8.8 Health Economics/Medical Resource Utilization and Health Economics

Health Economics/Medical Resource Utilization and Health Economics parameters are not evaluated in this study.

9 STATISTICAL CONSIDERATIONS

9.1 Statistical Hypotheses

The study hypothesis is that participants receiving pegvaliase will show a greater reduction in blood Phe following 72 weeks on study compared to the diet-only control group.

Let μ_a and μ_b denote the mean change from baseline in blood Phe following 72 weeks on study in the pegvaliase and diet-only group, respectively. The null and alternative hypotheses are:

H_0: $\mu_a = \mu_b$

H_1: $\mu_a \neq \mu_b$

The hypotheses will be tested at significance level of 0.05 (two-sided).

9.2 Sample Size Determination

Approximately 54 participants will be enrolled into the study and randomized to the active (pegvaliase) or control (diet-only) group in 2:1 ratio. Assuming a mean (SD) reduction in Phe following 72 weeks on study of 640 (570) µmol/L in the pegvaliase group and 100 (250) µmol/L in the diet-only group, a total sample size of 54 participants will provide more than 90% power to detect a treatment difference, based on the two-sample T-test with unequal variance and a significance level of 0.05 (two sided). The assumption of treatment effect is based on clinical data from previous pegvaliase studies and a PKU registry study. See the Statistical Analysis Plan (SAP) for additional details on the sample size calculation.

Approximately 72 participants will be screened for study participation such that approximately 54 participants will be enrolled in the study.

Note: Potential participants who are screened for the purpose of determining eligibility for the study, but do not participate in the study, are not considered enrolled.

9.3 Populations for Analysis

The analysis populations are defined in Table 9.3.1.

Table 9.3.1: Analysis Populations

Population	Description
All Enrolled	All participants (or their parent/guardians, if applicable) who agree to participate in the clinical study following completion of the informed consent process and determination of study eligibility.
ITT Population	All participants who are randomized.
Safety Population	All participants who received study treatment (pegvaliase or diet-only management of PKU).
Per Protocol Population	All participants in the ITT population who are compliant with the protocol with no major protocol deviations impacting the efficacy analysis. Major protocol deviations impacting efficacy analysis will be defined and documented in the SAP.
PK Population	All participants with at least one PK measurement

9.4 Statistical Analysis

The SAP will be finalized prior to database lock (DBL) and will include a more technical and detailed description of the statistical analyses described in this section. This section is a summary of the planned statistical analyses of the most important endpoints, including primary and key secondary endpoints.

A clinical pharmacology analysis plan will also be finalized prior to DBL.

Note: If discrepancies exist between the text of the statistical analysis as planned in the protocol, and the SAP, the SAP will prevail.

9.4.1 General Considerations

Descriptive analyses will be performed to gain an understanding of the qualitative and quantitative nature of the data collected and the characteristics of the sample collected. Continuous variables will be reported as mean, standard deviation (SD), median, minimum (min) and maximum (max). Categorical variables will be presented using absolute and relative frequency counts and percentage.

All generation of tables, listings and figures described in the SAP will be performed using SAS® version 9.4 or higher.

Full details on handling missing data and the corresponding analysis methods will be described in detail in the SAP.

9.4.2 Primary Endpoints

The primary efficacy endpoint is the change from baseline in blood Phe following 72 weeks on study. Baseline blood Phe is defined as the mean of 2 pre-treatment (Screening/Run-in)

measurements to be performed 7 to 10 days apart during the Screening/Run-in period. The blood Phe measurement following 72 weeks on study is defined as the average of the Week 69 and Week 73 blood Phe measurements.

Change from baseline will be compared between participants in the active (pegvaliase) arm and the control (diet-only) arm. The primary efficacy endpoint will be evaluated using an Analysis of Covariance (ANCOVA) model, which will include baseline blood Phe as covariate and treatment group as factor, with missing data handled by multiple imputation method. The least square (LS) mean and 95% confidence interval will be calculated for the treatment difference between pegvaliase and diet-only arms following 72 weeks on study. Additional imputation method may be conducted as sensitivity analysis, further details will be provided in the SAP.

9.4.3 Secondary Endpoints

The secondary endpoints are as follows:

- Change from baseline in total dietary protein intake (ie, medical food and/or intact food). Baseline total protein intake is defined as the mean total dietary protein intake calculated from the 3 separate 3-day diet diaries collected during Screening/Run-in. The total dietary protein intake following 72 weeks on study is defined as the mean total dietary protein intake calculated from the diet diaries collected at the Week 69 and Week 73 visits. Total dietary protein is measured in grams/kilogram (g/kg).
- Plasma sample for trough PK

Descriptive summary will be provided. Full details will be provided in the SAP.

9.4.4 Exploratory Endpoints

Refer to the SAP for a discussion of analysis for exploratory endpoints.

9.4.5 Other Safety Analyses

Safety analyses will be performed on the Safety Population. AEs will be coded using the most recent version of the Medical Dictionary for Regulatory Activities (MedDRA). The incidence of treatment-emergent AEs, SAEs, and AESIs will be summarized by MedDRA system organ class, preferred term, relationship to study drug, and severity. Participants with AEs that result in study drug discontinuation, dosing interruption, or dose level reduction will be tabulated. A by-participant listing of all AEs will be provided.

Clinical laboratory data will be summarized descriptively at baseline and post-baseline visits. Shift tables will be created to summarize the change in CTCAE grade from baseline to worst post-baseline value. Clinically significant laboratory abnormalities reported as AEs will be

summarized. Descriptive statistics for vital signs, physical examination results, ECG test results, and immunogenicity test results will also be provided. Details of statistical methods will be provided in the SAP.

9.5 Interim Analyses

No interim analyses are planned for this study.

9.6 Data Monitoring Committee (DMC)

The independent Data Monitoring Committee (DMC) will act in an advisory capacity to BioMarin to monitor participant safety and the efficacy of pegvaliase in participants who participate in Study 165-306. The DMC responsibilities may include:

- Review the study protocol, informed consent and assent documents, and plans for data monitoring
- Evaluate the progress of the trial, study data quality, timeliness, participant recruitment, accrual and retention, participants' risk versus benefit, and other factors that could affect the study outcome
- Consider relevant information that may have an impact on the safety of the participants or the ethics of the study
- Protect the safety of the study participants in accordance with the stopping rules as defined in study protocol
- Make recommendations to BioMarin concerning continuation, termination or other modifications of the study based on their observations of the study

Further details are provided in the DMC Charter.

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 Appendix 1: Regulatory, Ethical, and Study Oversight Considerations

10.1.1 Regulatory and Ethical Considerations

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

- Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines
- Principles of Good Clinical Practice as described in the US Code of Federal Regulations and the International Conference on Harmonisation Guidelines
- Applicable laws and regulations

The protocol, protocol amendments, ICF, Investigator Brochure, and other relevant documents (eg, advertisements) as applicable must be submitted, reviewed, and approved by the IRB/IEC before the study is initiated.

Any amendments to the protocol will require IRB/IEC approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study participants.

The investigator will be responsible for the following:

- Providing written summaries of the status of the study to the IRB/IEC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/IEC
- Notifying the IRB/IEC of SAEs or other significant safety findings as required by IRB/IEC procedures
- Providing oversight of the conduct of the study at the site and adherence to requirements of 21 CFR, ICH guidelines, the IRB/IEC, European regulation 536/2014 for clinical studies (if applicable), and all other applicable local regulations

BioMarin will pay the full costs of the study-related tests, procedures, and treatments set forth in this protocol. In addition, after IRB/IEC approval, BioMarin may reimburse the reasonable cost of travel for study-related visits in accordance with BioMarin's travel and reimbursement policy.

The investigator should contact BioMarin immediately upon notification that a study participant has been injured by the study drug or by procedures performed as part of the study. Any participant who experiences a study-related injury should be instructed by the

investigator to seek immediate medical treatment at a pre-specified medical institution if possible, or at the closest medical treatment facility if necessary. The participant should be given the name of a person to contact to seek further information about, and assistance with, treatment for study-related injuries. BioMarin or the institution may pay for reasonable and necessary medical services to treat the injuries caused by the study drug or study procedures. In some jurisdictions, BioMarin is obligated by law to pay for study-related injuries. If this is the case, BioMarin will comply with the law. BioMarin will not pay for any hospitalizations, tests, or treatments for medical problems of any sort related solely to the study participant's primary disease or any concurrent disease and that are unrelated to the study.

Significant Noncompliance Reporting: Any deviation or series of deviations from the Protocol, ICH GCP, regulatory, and/or legal requirements that is likely to affect to a significant degree the safety or rights of a participant; the stability, quality, and/or control of study drug; or the quality or integrity of the study data shall be reported in writing to BioMarin within one (1) business day upon becoming aware of any suspicion or discovery of Significant Noncompliance.

10.1.2 Statement of Investigator and Financial Disclosure

Prior to beginning the study, the investigator at each site must provide to sponsor or designee a fully executed and signed Statement of Investigator (SOI) form. A US Food and Drug Administration (FDA) Form FDA 1572 serves as an acceptable SOI form. If Form FDA 1572 may not be used in a particular region, the investigator must provide a fully executed SOI on the form provided by the sponsor. All investigators and sub-investigators must be listed on Form FDA 1572 or its equivalent SOI. Financial Disclosure Forms must be completed with sufficient, accurate financial information as requested for all investigators and sub-investigators listed on the Form FDA 1572 or SOI who will be directly involved in the treatment or evaluation of participants in this study. The Financial Disclosure Forms allow the sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities. Investigators must submit an updated Financial Disclosure Form to BioMarin should their response to any questions within the form or disclosure status change during the period of the study and for one year after study completion.

In accordance with FDA Form 1572 and/or principles of ICH GCP, the investigator will ensure that:

- He or 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 participants.

- He or she will personally conduct or supervise the study.
- He or she will inform any potential participants, 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 CFR Part 50 and/or ICH Sections 2.9 and 4.8 are met. As well, he or she will ensure that IRB/IEC review and approval in 21 CFR Part 56 and/or ICH Section 2.6 are met.
- He or she will report to the sponsor adverse experiences that occur in the course of the investigation in accordance with 21 CFR 312.64 and/or ICH Section 4.11.
- He or she has read and understands the information in the IB, including potential risks and side effects of the drug.
- His or her staff and all persons who assist in the conduct of the study are informed about their obligations in meeting the above commitments.
- Adequate and accurate records in accordance with 21 CFR 312.62 and/or ICH Section 4.9 are kept, and those records are available for inspection in accordance with 21 CFR 312.68 and/or ICH Section 4.9.7.
- The IRB/EC/REB complies with the requirements of 21 CFR Part 56, ICH Section 3.0, and other applicable regulations, and conducts initial and ongoing reviews and approvals of the study. He or she will also ensure that any change in research activity and all problems involving risks to human participants or others are reported to the IRB/EC/REB. Additionally, he or she will not make any changes in the research without IRB/EC/REB approval, except where necessary to eliminate apparent immediate hazards to human participants.
- He or she agrees to comply with all other requirements regarding the obligations of clinical Investigators and all other pertinent requirements in 21 CFR Part 312 and/or ICH E6 R2.

10.1.3 Informed Consent Process

The investigator or his/her representative will explain the nature of the study to the participant or his/her legally authorized representative and answer all questions regarding the study.

Participants must be informed that their participation is voluntary. Participants or their legally authorized representative (as determined by the laws of the jurisdiction in which the study is being conducted) will be required to sign a statement of informed consent that meets the regulatory requirements of the country or region where the participant consents, such as 21 CFR 50 (US FDA), ICH guidelines, privacy requirements including but not limited to the Health Insurance Portability and Accountability Act (HIPAA) requirements and the General Data Protection Regulation (where applicable), and the IRB/IEC or study center. If there is no applicable law addressing the issue of who may be a legally authorized representative, a

legally authorized representative will be an individual recognized by institutional policy as acceptable for providing consent on behalf of the prospective participant to the participant's participation in the procedure(s) involved in the research.

The medical record must include a statement that written informed consent was obtained before any study specific procedures were initiated and the date the written consent was obtained. The authorized person obtaining the informed consent must also sign the ICF.

Participants must be re-consented to the most current version of the ICF(s) during their participation in the study. Participants who reach the age of majority in their country while the study is ongoing will be asked to provide their own written consent again upon reaching the legal age of majority.

The ICF will contain a separate section that addresses the use of remaining samples for optional additional research. The investigator or authorized designee will explain to each participant the objectives of the additional research. Participants will be told that they are free to refuse to participate and may withdraw their consent at any time and for any reason during the storage period. A separate signature will be required to document a participant's agreement to allow any remaining samples to be used for additional research.

A copy of the ICF(s) must be provided to the participant or the participant's legally authorized representative.

A participant who is rescreened is not required to sign another ICF if the rescreening occurs during the Screening/Run-in period.

10.1.4 Data Protection

Measures will be taken to ensure the privacy of participant data, including the use of participant numbers on eCRFs. A list linking participant identification numbers with participant names and other personal information will be kept in a secure place, separate from the participants' medical records. BioMarin will follow all applicable regulations and guidelines in the relevant locality, country, and/or region to protect the privacy of participant data.

Participants will be assigned a unique identifier by the sponsor. Any participant records or datasets that are transferred to the sponsor will contain the identifier only; participant names or any information which would make the participant identifiable will not be transferred.

The participant must be informed that his/her personal study-related data will be used by the sponsor in accordance with local data protection law. The level of disclosure must also be

explained to the participant, who will be required to give consent or be given notice of the legal bases for their data to be used as described in the informed consent.

The participant must be informed that his/her medical records may be examined by authorized personnel appointed by the sponsor, by appropriate IRB/IEC members, and by inspectors from regulatory authorities.

10.1.5 Committee Structures

The following advisory and assurance activities will be implemented to support the study: monitoring committees, clinical trial disclosure, and study monitoring.

10.1.5.1 Data Monitoring Committee (DMC)

An external independent Data Monitoring Committee (DMC) will be responsible for monitoring participant safety and efficacy of pegvaliase. The DMC consisting of five members, who are each independent of the conduct of studies in the BioMarin BMN 165 (pegvaliase) development program, and are experts in the fields of PKU, clinical immunology, neuropsychology, pharmacovigilance, and biostatistics. DMC responsibilities are described in Section 9.6.

10.1.5.2 Internal Safety Data Review Board

The Internal Safety Data Review Board, consisting of a representative from BioMarin clinical sciences, safety, and biostatistics, will review all cases of potential anaphylaxis to determine which of these events meet the criteria for an acute systemic hypersensitivity reaction as summarized in Appendix 5, Section 10.5.2.

10.1.6 Dissemination of Clinical Study Data

The posting of study information and study results will comply with applicable national regulatory requirements and BioMarin's data sharing policy available at https://www.biomarin.com/data-request-form/.

10.1.7 Data Quality Assurance

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

Sites will enter study data into eCRFs in the study EDC system. Data Quality Control will be performed by BioMarin Clinical Data Management or a designee through implementation of

quality control checks specified in the study data management plan and edit check specifications.

Study monitors will perform ongoing source data verification to confirm that data entered into the eCRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of participants are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements. Qualified individuals designated by BioMarin will monitor all aspects of the study according to 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 participants, 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 BioMarin or its designees.

The investigator must retain all study records required by BioMarin and by the applicable regulations in a secure and safe facility. The investigator must notify BioMarin 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, that is, 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, participant 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) at least 2 years have elapsed since the formal discontinuation of clinical development of the IP. The investigator/institution should retain participant identifiers for at least 15 years after the completion or discontinuation of the study. Participant 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 a BioMarin agreement. BioMarin must be notified and will assist with retention should the investigator/institution be unable to continue maintenance of participant files for the full 15 years. It is the responsibility of BioMarin to inform the investigator/institution as to when these documents no longer need to be retained.

Representatives of BioMarin or designees may conduct an audit of a site at any time before, 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 inspection of the study. If informed of such an inspection, the investigator should notify BioMarin immediately. The investigator will ensure that the auditors/inspectors have access to the clinical supplies, site facilities, original source documentation, and all study files.

10.1.8 Source Documents

Source documents provide evidence for the existence of the participant and substantiate the integrity of the data collected. Source documents are filed at the investigator's site.

Data entered in the eCRF that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The investigator may need to request previous medical records or transfer records, depending on the study. Also, current medical records must be available.

The definition of what constitutes source data can be found in the Monitoring Plan.

10.1.9 Study and Site Start and Closure

First Act of Recruitment

The study start date is the date on which the clinical study will be open for recruitment of participants.

The first act of recruitment is the first site open and will be the study start date.

Study/Site Termination

The sponsor reserves the right to close the site or terminate the study at any time for any reason at the sole discretion of the sponsor. Sites will be closed upon study completion. A site is considered closed when all required documents and unused study supplies have been collected and a site closure visit has been performed.

Reasons for the early closure of a site by the sponsor may include but are not limited to:

- Failure of the investigator to comply with the protocol, the requirements of the IRB/IEC or local health authorities, the sponsor's procedures, or GCP guidelines
- Inadequate recruitment of participants by the investigator
- Discontinuation of further study treatment development

If the study is prematurely terminated or suspended, the sponsor shall promptly inform the investigators, the IECs/IRBs, the regulatory authorities, and any contract research

organization(s) used in the study of the reason for termination or suspension, as specified by the applicable regulatory requirements. The investigator shall promptly inform the participant(s) and should assure appropriate participant therapy and/or follow-up.

10.1.10 Publication Policy

The results of this study may be published or presented at scientific meetings. If this is foreseen, the investigators agree to submit all manuscripts or abstracts to the sponsor at least forty-five (45) days before submission or as agreed in the Clinical Trial Agreement between BioMarin and the investigator/institution. This allows the sponsor to protect proprietary information and to provide comments.

The sponsor will comply with the requirements for publication of study results. In accordance with standard editorial and ethical practice, the sponsor will generally support publication of multicenter studies only in their entirety and not as individual site data. In this case, a coordinating investigator will be designated by mutual agreement.

BioMarin recognizes the importance of communicating medical study data and therefore encourages the publication of these data 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 may be described in the Clinical Trial Agreement between BioMarin and the investigator/institution. Consideration for authorship of all publications will be based on mutual agreement and compliance with the Uniform Requirements for Manuscripts Submitted to Biomedical Journals (“Uniform Requirements”) of the International Committee of Medical Journal Editors (ICMJE) (http://www.icmje.org/about-icmje/faqs/icmje-recommendations/) and good publication practices (GPP).

10.2 Appendix 2: Clinical Laboratory Tests

The tests detailed in Table 10.2.1 will be performed by the central laboratory.

Protocol-specific requirements for inclusion or exclusion of participants are detailed in Section 5 of the protocol.

Additional tests may be performed at any time during the study as determined necessary by the investigator or required by local regulations.

Investigators must document their review of each laboratory safety report.

Table 10.2.1: Clinical Laboratory Tests

Laboratory Test	Parameters		
Chemistry	Albumin Alkaline Phosphatase ALT (SGPT) AST (SGOT) Bicarbonate Direct bilirubin Total bilirubin	BUN Calcium Total cholesterol CO_2 Creatinine Creatine phosphokinase Glucose (fasting)	GGT LDH Phosphorus Potassium Total protein Sodium Uric acid
Hematology	Hemoglobin Hematocrit	WBC count RBC count	Platelet count Differential cell count
Urinalysis	Appearance Color pH Specific gravity Ketone Protein	Glucose Bilirubin Nitrite Urobilinogen Hemoglobin	Urinary albumin/creatinine ratio [a] Urinary microscopy
Pregnancy testing	Refer to Section 8.2.5		
Complement panel	C3 C4		
Other tests conducted during the study (refer to the SoA for timing)	Phenylalanine Tyrosine hs-CRP Serum tryptase level Serum cortisol [b] Erythrocyte sedimentation rate [c] Urinary N-methylhistamine		

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; C3, C4, complement components 3, 4; CO2, carbon dioxide; ESR, Erythrocyte sedimentation rate; GGT, gamma-glutamyltransferase; hs-CRP, high sensitivity C-reactive protein; LDH, lactate dehydrogenase; RBC, red blood cell; SGOT, serum glutamic-oxaloacetic transaminase; SGPT, serum glutamic-pyruvic transaminase; WBC, white blood cell.

[a] It is recommended that urine samples be obtained as a first or second morning void. Participants with a confirmed urine/albumin creatinine ratio of ≥ 100 mg/g should be referred to a nephrologist for consultation if results were within normal range at Baseline. Participants who had elevated results at Baseline followed by a confirmed subsequent increase of 100-200 mg/g from Baseline should also be referred to a nephrologist for consultation.

[b] Serum cortisol samples should be taken before study drug administration in the morning before 10:00 am. A home healthcare nurse may collect samples for participant convenience. If 2 results are low and abnormal, the participant will be asked to perform additional sampling for serum cortisol and a low-dose conventional ACTH stimulation test or the ACTH test method based on the clinical practice at the site.

[c] ESR will be performed at a local laboratory.

10.3 Appendix 3: Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting

10.3.1 Definition of an Adverse Event

AE Definition
• An AE is any untoward medical occurrence in a patient or clinical study participant, temporally associated with the use of study treatment, whether or not considered related to the study treatment. • NOTE: An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of study treatment.

Events Meeting the AE Definition
• Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (eg, ECG, radiological scans, vital signs measurements), including those that worsen from baseline, considered clinically significant in the medical and scientific judgment of the investigator (ie, not related to progression of underlying disease). • Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition. • New conditions detected or diagnosed after study treatment administration. • Signs, symptoms, or the clinical sequelae of a suspected drug-drug interaction. • Signs, symptoms, or the clinical sequelae of a suspected overdose of either study treatment or a concomitant medication. Overdose per se will not be reported as an AE/SAE unless it is an intentional overdose taken with possible suicidal/self-harming intent. Such overdoses should be reported regardless of sequelae. • "Lack of efficacy" or "failure of expected pharmacological action" per se will not be reported as an AE or SAE. Such instances will be captured in the efficacy assessments. However, the signs, symptoms, and/or clinical sequelae resulting from lack of efficacy will be reported as AE or SAE if they fulfill the definition of an AE or SAE.

Events Not Meeting the AE Definition
• Any clinically significant abnormal laboratory findings or other abnormal safety assessments which are associated with the underlying disease, unless judged by the investigator to be more severe than expected for the participant's condition. • The disease/disorder being studied or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the participant's condition. • Medical or surgical procedure (eg, endoscopy, appendectomy): the condition that leads to the procedure is the AE. • Situations in which an untoward medical occurrence did not occur (social and/or convenience admission to a hospital).

Events Not Meeting the AE Definition
• Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen. • Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline. • Planned hospitalization for childbirth (eg, scheduled Caesarean section).

10.3.2 Definition of an SAE

If an event is not an AE per the definition above, then it cannot be an SAE even if serious conditions are met (eg, hospitalization for signs/symptoms of the disease under study, death due to progression of disease).

A SAE is defined as any untoward medical occurrence that, at any dose:
a. Results in death
b. Is life-threatening The term "life-threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event. It does not refer to an event that hypothetically might have caused death if it were more severe.
c. Requires inpatient hospitalization or prolongation of existing hospitalization In general, hospitalization signifies that the participant has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious.
d. Results in persistent disability/incapacity The term disability means a substantial disruption of a person's ability to conduct normal life functions. This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (eg, sprained ankle) which may interfere with or prevent everyday life functions but do not constitute a substantial disruption.
e. Is a congenital anomaly/birth defect in the offspring of a study participant
f. Other situations/Important Medical Events: Medical or scientific judgment should be exercised in deciding whether SAE reporting is appropriate in other situations such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the participant or may require medical or surgical treatment to prevent one of the other outcomes listed in the above definition. These events should usually be considered serious. Examples of such events include invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

10.3.3 Recording and Follow-Up of AEs and/or SAEs

AE and SAE Recording

- When an AE/SAE occurs, it is the responsibility of the investigator to review all documentation (eg, hospital progress notes, laboratory reports, and diagnostics reports) related to the event.
- The investigator or qualified designee will then record all relevant AE/SAE information in the eCRF.
- It is **not** acceptable for the investigator to send photocopies of the participant's medical records to BioMarin in lieu of completion of the AE/SAE eCRF page.
- There may be instances when copies of medical records for certain cases are requested by BioMarin. In these cases, all participant identifiers, with the exception of the participant number, will be redacted on the copies of the medical records before submission to BioMarin.
- The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. Whenever possible, the diagnosis (not the individual signs/symptoms) will be documented as the AE/SAE.

Assessment of Intensity

The investigator will make an assessment of intensity for each AE and SAE reported during the study and assign it to 1 of the following categories (as defined in Common Terminology Criteria for Adverse Events [CTCAE] version 5.0). The investigator should only record the single highest grade of intensity for each AE and SAE in the eCRF. Adverse events that do not have a corresponding CTCAE term will be assessed according to the general guidelines for grading:

- Grade 1 (Mild): asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated
- Grade 2: (Moderate): minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL) (eg, preparing meals, shopping for groceries or clothes, using the telephone, managing money)
- Grade 3 (Severe): medically significant but not immediately life-threatening: hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL (eg, bathing, dressing/undressing, feeding oneself, using the toilet, taking medications, not bedridden)
- Grade 4: Life threatening consequences; urgent intervention indicated
- Grade 5: Death related to AE

An event is defined as "serious" when it meets at least 1 of the predefined outcomes as described in the definition of an SAE, NOT when it is rated as severe.

Assessment of Causality

- The investigator is obligated to assess the relationship between study treatment and each occurrence of each AE/SAE.
- An assessment of "related" means that there are facts, evidence, and/or arguments to suggest a causal relationship such that a relationship cannot be ruled out.
- The investigator will use clinical judgment to determine the relationship.

Assessment of Causality
• Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to study treatment administration will be considered and investigated. • For each AE/SAE, the investigator must document in the medical notes that he/she has reviewed the AE/SAE and has provided an assessment of causality. • There may be situations in which an SAE has occurred, and the investigator has minimal information to include in the initial report to BioMarin. However, it is very important that the investigator always make an assessment of causality for every event before the initial transmission of the SAE data to BioMarin. • The investigator may change his/her opinion of causality in light of follow-up information and send a SAE follow-up report with the updated causality assessment.

Follow-up of AEs and SAEs
• The investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by BioMarin to elucidate the nature and/or causality of the AE or SAE as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals. • If a participant dies during participation in the study or during a recognized follow-up period, the investigator will provide a copy of any post-mortem findings including histopathology. • New or updated information will be recorded in the originally completed eCRF. • The investigator will submit any updated SAE data to the sponsor within 24 hours of receipt of the information.

10.3.4 Reporting of SAEs and Events of Special Interest

SAE/AESI Reporting to BioMarin Pharmacovigilance (BPV) via an Electronic Data Collection Tool
• The primary mechanism for reporting an SAE/AESI to BPV will be the electronic data collection tool. AESI reporting will follow the SAE reporting requirements. • If the electronic system is unavailable, then the site will use the paper SAE data collection tool (see next section) in order to report the event within 24 hours. • The site will enter the SAE/AESI data into the electronic system as soon as it becomes available. • After the study is completed at a given site, the electronic data collection tool will be taken off-line to prevent the entry of new data or changes to existing data. • If a site receives a report of a new SAE/AESI from a study participant or receives updated data on a previously reported SAE/AESI after the electronic data collection tool has been taken off-line, then the site can report this information on a paper SAE form (see next section) or to BPV by telephone.

SAE/AESI Reporting to BPV via Paper CRF
• Facsimile transmission or email of the paper CRF (pregnancy form, pregnancy outcome, or SAE form is EDC unavailable) is the preferred method to transmit this information to BPV. • In rare circumstances and in the absence of facsimile equipment, notification by telephone is acceptable with a copy of the paper CRF sent by overnight mail or courier service. • Initial notification via telephone does not replace the need for the investigator to complete and sign the CRF pages within the designated reporting time frames.

Contact information for BioMarin Pharmacovigilance is as follows:

BioMarin Pharmaceutical Inc.
Address 105 Digital Drive
Novato, CA 94949
Phone: PI
Fax:
E-mail: drugsafety@bmrn.com

The Investigator is encouraged to discuss with the medical monitor any AEs for which the issue of seriousness is unclear or questioned. Contact information for the medical monitor is provided on the signature page at the front of this protocol.

Name: PI RN, MS
Address: 105 Digital Drive
Novato, CA 94949 USA
Phone: PI
Fax:
E-mail:

10.4 Appendix 4: Contraceptive and Barrier Guidance

Definitions:

Woman of Childbearing Potential (WOCBP)

A woman is considered fertile following menarche and until becoming post-menopausal unless permanently sterile (see below).

If fertility is unclear (eg, amenorrhea in adolescents or athletes) and a menstrual cycle cannot be confirmed before first dose of study treatment, additional evaluation should be considered.

Women in the following categories are not considered WOCBP:

- Premenarchal
- Premenopausal female with 1 of the following:
    - Documented hysterectomy
    - Documented bilateral salpingectomy
    - Documented bilateral oophorectomy

  For individuals with permanent infertility due to a medical cause other than the above (eg, mullerian agenesis, androgen insensitivity), investigator discretion should be applied in determining study eligibility.

  Note: Documentation can come from the site personnel's review of the participant's medical records, medical examination, or medical history interview.
- Postmenopausal female
    - A postmenopausal state is defined as no menses for 12 months without an alternative medical cause.
        - A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a postmenopausal state in women not using hormonal contraception or hormonal replacement therapy (HRT). However, in the absence of 12 months of amenorrhea, confirmation with more than one FSH measurement is required.
    - Females on HRT whose menopausal status is in doubt will be required to use one of the non-estrogen hormonal highly effective contraception methods if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of postmenopausal status before study enrollment.

Contraception Guidance:

If sexually active, must be willing to use two acceptable methods of contraception while participating in the study and 4 weeks after the last dose of pegvaliase.

10.5 Appendix 5: Anaphylaxis Criteria

For the purposes of this study, the term anaphylaxis refers to the important identified risk of acute systemic hypersensitivity reaction. An acute systemic hypersensitivity reaction is one that is consistent with the NIAID/FAAN definition as assessed by BioMarin using the criteria listed below in Appendix 5, Section 10.5.1 and Appendix 5, Section 10.5.2.

10.5.1 Sampson's Anaphylaxis Criteria

According to the National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network (NIAID/FAAN) Second Symposium on the definition and management of anaphylaxis, anaphylaxis is highly likely when any one of the following 3 criteria are fulfilled:

1. Acute onset of an illness (minutes to several hours) with involvement of the skin, mucosal tissue, or both (eg, generalized hives, pruritus or flushing, swollen lips-tongue-uvula)

   AND AT LEAST ONE OF THE FOLLOWING:

   a. Respiratory compromise (eg, dyspnea, wheeze-bronchospasm, stridor, reduced peak expiratory flow [PEF], hypoxemia)

   b. Reduced blood pressure (BP) or associated symptoms of end-organ dysfunction (eg, hypotonia [collapse], syncope, incontinence)

2. Two or more of the following that occur rapidly after exposure *to a likely allergen for that patient* (minutes to several hours):

   a. Involvement of the skin-mucosal tissue (eg, generalized hives, itch-flush, swollen lips-tongue-uvula)

   b. Respiratory compromise (eg, dyspnea, wheeze-bronchospasm, stridor, reduced PEF, hypoxemia)

   c. Reduced BP or associated symptoms (eg, hypotonia [collapse], syncope, incontinence)

   d. Persistent gastrointestinal symptoms (eg, crampy abdominal pain, vomiting)

3. Reduced BP after exposure to *known* *allergen for that patient* (minutes to several hours):
    a. Infants and children: low systolic blood pressure (age specific) or greater than 30% decrease is systolic BP
    b. Adults: systolic BP of less than 90 mmHg or greater than 30% decrease from that person's baseline.

Source: Sampson 2006.

10.5.2 Acute Systemic Hypersensitivity Reaction

An acute systemic hypersensitivity reaction is a Type III acute systemic allergic reaction. An acute systemic hypersensitivity reaction has a similar clinical presentation to an anaphylactic reaction, but only meets the definition if the italicized criteria below are met in addition to the criteria outlined in Sampson's criteria (see Appendix 5, Section 10.5.1, Sampson's Criteria):

Sampson Criteria Number	Sampson Criteria Wording With Additional Criteria Necessary to Meet Acute Systemic Hypersensitivity Reaction Criteria Shown in Italics
1	Acute onset of an illness (minutes to several hours) with involvement of the skin, mucosal tissue, or both (eg, generalized hives, pruritus or flushing, swollen lips-tongue-uvula). *Must be generalized skin reaction to meet criteria*.
1a	Respiratory compromise (eg, dyspnea, wheeze-bronchospasm, stridor, reduced PEF, hypoxemia). *Respiratory compromise is demonstrated by oxygen desaturation below 88% on room air.*
1b	Reduced BP or associated symptoms of end-organ dysfunction (eg, hypotonia [collapse], syncope, incontinence). *Systolic BP less than 90 mmHg (adults) or 70 mmHg (children) or greater than 30% decrease from baseline*.
2d	Persistent gastrointestinal symptoms (eg, crampy abdominal pain, vomiting). *Gastrointestinal symptoms are persistent with multiple episodes that continue for several hours or more*.

10.5.3 Anaphylaxis Analysis

During the initial BLA for Palynziq in the US, the FDA proposed that anaphylaxis should be defined for labelling purposes using the methodology developed by FDA's Division of Pulmonary, Allergy, and Rheumatology Products (DPARP). As a result, in the study analyses for 165-306 anaphylaxis, including derivation of the incidence and event rates, data will be presented using both BioMarin's definition of an acute systemic hypersensitivity reaction described above and the FDA's assessment methodology, ie, a case will be counted as an anaphylaxis event if it meets any of the following criteria:

- Clinical manifestations consistent with NIAID/FAAN criterion #1 (skin involvement + respiratory or cardiovascular compromise); or
- Verbatim reports of anaphylaxis/anaphylactic reaction (or synonyms), even if NIAID/FAAN criteria were not met; or
- Cases involving participant injection of epinephrine alone, even if the participant did not experience signs/symptoms necessary to meet NIAID/FAAN criteria.

Further details are provided in the SAP.

10.6 Appendix 6: Medical Device Adverse Events, Adverse Device Effects (ADEs), Serious Adverse Events (SAEs), and Device Deficiencies: Definition and Procedures for Recording, Evaluating, Follow-Up, and Reporting

Medical Device Adverse Event reporting applies only to US sites. The definitions and procedures detailed in this appendix are in accordance with ISO 14155.

Both the investigator and the sponsor will comply with all local medical device reporting requirements.

The detection and documentation procedures described in this protocol apply to all sponsor medical devices provided for use in the study. For this study, prefilled syringes are provided (see Section 6.1.1).

10.6.1 Definition of AE and ADE

AE and ADE Definition
• An AE is defined as any untoward medical occurrence, unintended disease or injury, or untoward clinical signs (including abnormal laboratory finding) in study participants, users, or other persons, whether or not related to the investigational medical device. This definition includes events related to the investigational medical device or comparator and events related to the procedures involved except for events in users or other persons, which only include events related to investigational devices. • An adverse device effect (ADE) is defined as an adverse event related to the use of an investigational medical device. This definition includes any adverse events resulting from insufficient or inadequate instructions for use, deployment, implantation, installation, or operation, or any malfunction of the investigational medical device as well as any event resulting from use error or from intentional misuse of the investigational medical device.

10.6.2 Definition of SAE, SADE, and USADE

If an event is not an AE per definition above, then it cannot be an SAE even if serious conditions are met (eg, hospitalization for signs/symptoms of the disease under study, death due to disease progression).

An SAE is an AE that:

- Led to death
- Led to serious deterioration in the health of the participant resulting in either:
  1. A life-threatening illness or injury. The term 'life-threatening' in the definition of 'serious' refers to an event in which the participant was at risk of death at the time of the event. It does not refer to an event which hypothetically might have caused death if it were more severe.
  2. A permanent impairment of a body structure or a body function
  3. Inpatient or prolonged hospitalization, planned hospitalization for a pre-existing condition, or a procedure required by the protocol, without serious deterioration in health, is not considered an SAE.
  4. Medical or surgical intervention to prevent life-threatening illness or injury or permanent impairment to a body structure or a body function
- Led to fetal distress, fetal death, or a congenital abnormality or birth defect

SADE definition:

- A SADE is defined as an adverse device effect that has resulted in any of the consequences characteristic of a serious adverse event.

USADE definition

- A USADE is a serious adverse device effect which by its nature, incidence, severity, or outcome has not been identified in the current version of the risk analysis report.

10.6.3 Definition of Device Deficiency

Device deficiency definition

- A device deficiency is an inadequacy of a medical device with respect to its identity, quality, durability, reliability, safety, or performance. Device deficiencies include malfunctions, use errors, and inadequate labeling.

10.6.4 Recording and Follow-Up of AE and/or SAE and Device Deficiencies

AE, SAE, and Device Deficiency Recording

- When an AE/SAE/device deficiency occurs, it is the responsibility of the investigator to review all documentation (e.g., hospital progress notes, laboratory reports, and diagnostics reports) related to the event.
- The investigator will then record all relevant AE/SAE/device deficiency information in the participant's medical records, in accordance with the investigator's normal clinical practice and on the appropriate form of the eCRF.
- All ADEs will be entered into the AE eCRF.
- SADEs will be entered into the AE eCRF and submitted to BPV. The site will also complete and submit the study specific paper device report form within 24 hours of site awareness.
- It is not acceptable for the investigator to send photocopies of the participant's medical records to BPV in lieu of completion of the /AE/SAE/device deficiency eCRF page.
- There may be instances when copies of medical records for certain cases are requested by BPV. In this case, all participant identifiers, with the exception of the participant number, will be redacted on the copies of the medical records before submission to BPV.
- The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. Whenever possible, the diagnosis (not the individual signs/symptoms) will be documented as the AE/SAE.
- For device deficiencies, it is very important that the investigator describes any corrective or remedial actions taken to prevent recurrence of the incident.
- A remedial action is any action other than routine maintenance or servicing of a medical device where such action is necessary to prevent recurrence of a device deficiency. This includes any amendment to the device design to prevent recurrence.

Assessment of Intensity

- The investigator will make an assessment of intensity for each AE/SAE/device deficiency reported during the study and assign it to one of the following categories:
  - Mild: An event that is easily tolerated by the participant, causing minimal discomfort and not interfering with everyday activities.
  - Moderate: An event that causes sufficient discomfort and interferes with normal everyday activities.
  - Severe: An event that prevents normal everyday activities. An AE that is assessed as severe should not be confused with a SAE. Severe is a category utilized for rating the intensity of an event; and both AEs and SAEs can be assessed as severe.
- An event is defined as 'serious' when it meets at least 1 of the predefined outcomes as described in the definition of an SAE, NOT when it is rated as severe.

Assessment of Causality

- The investigator is obligated to assess the relationship between study treatment and each occurrence of each AE/SAE/device deficiency.
- A "reasonable possibility" of a relationship conveys that there are facts, evidence, and/or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out.
- The investigator will use clinical judgment to determine the relationship.
- Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to study treatment administration will be considered and investigated.
- The investigator will also consult the IB in his/her assessment.
- For each AE/SAE/device deficiency, the investigator must document in the medical notes that he/she has reviewed the AE/SAE/device deficiency and has provided an assessment of causality.
- There may be situations in which an SAE has occurred and the investigator has minimal information to include in the initial report to BPV. However, it is very important that the investigator always make an assessment of causality for every event before the initial transmission of the SAE data to BPV.
- The investigator may change his/her opinion of causality in light of follow-up information and send a SAE follow-up report with the updated causality assessment.
- The causality assessment is one of the criteria used when determining regulatory reporting requirements.

Follow-up of AE/SAE/device deficiency

- The investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by BPV to elucidate the nature and/or causality of the AE/SAE/device deficiency as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.
- If a participant dies during participation in the study or during a recognized follow-up period, the investigator will provide BPV with a copy of any post mortem findings including histopathology.
- New or updated information will be recorded in the originally completed eCRF.
- The investigator will submit any updated SAE data to BPV within 24 hours of receipt of the information.

10.6.5 Reporting of SAEs

SAE Reporting to BPV via an Electronic Data Collection Tool

- The primary mechanism for reporting an SAE to BPV will be the electronic data collection tool.
- If the electronic system is unavailable, then the site will use the paper SAE data collection tool (see next section) in order to report the event within 24 hours.
- The site will enter the SAE data into the electronic system as soon as it becomes available.
- After the study is completed at a given site, the electronic data collection tool will be taken off-line to prevent the entry of new data or changes to existing data.
- If a site receives a report of a new SAE from a study participant or receives updated data on a previously reported SAE after the electronic data collection tool has been taken off-line, then the site can report this information on a paper SAE form (see next section) or to BPV by telephone.
- Contacts for SAE reporting can be found in Appendix 3, Section 10.3.4.

SAE Reporting to BPV via Paper CRF

- Facsimile transmission of the SAE paper CRF is the preferred method to transmit this information to BPV.
- In rare circumstances and in the absence of facsimile equipment, notification by telephone is acceptable with a copy of the SAE data collection tool sent by overnight mail or courier service.
- Initial notification via telephone does not replace the need for the investigator to complete and sign the SAE CRF pages within the designated reporting time frames.
- Contacts for SAE reporting can be found in Appendix 3, Section 10.3.4.

10.6.6 Reporting of SADEs

SAE Reporting to BPV

NOTE: There are additional reporting obligations for medical device incidents that are potentially related to SAEs that must fulfil the legal responsibility to notify appropriate regulatory authorities and other entities about certain safety information relating to medical devices being used in clinical studies.

- Any device deficiency that is associated with an SAE must be reported to the sponsor within 24 hours after the investigator determines that the event meets the definition of a device deficiency.
- The sponsor shall review all device deficiencies and determine and document in writing whether they could have led to an SAE. These shall be reported to the regulatory authorities and IRBs/IECs as required by national regulations.
- Contacts for SAE reporting can be found in Appendix 3, Section 10.3.4.

10.7 Appendix 7: Abbreviations

Abbreviation	Definition
ACMG	American College of Medical Genetics and Genomics
ADHD	Attention Deficit Hyperactivity Disorder
ADHD RS-IV	Attention Deficit Hyperactivity Disorder Rating Scale
ADL	activities of daily living
AE	adverse event
AESI	adverse events of special interest
ALT	alanine aminotransferase
ANCOVA	Analysis of Covariance
AST	aspartate aminotransferase
AUC	area under the concentration-time curve
BH4	tetrahydrobiopterin
BLA	Biologics License Application
BPV	BioMarin Pharmacovigilance
BRIEF2	Behavior Rating Inventory of Executive Function, Second Edition
C3	complement component 3
C4	complement component 4
CaGIC	Caregiver Global Impression of Change
CaGIS	Caregiver Global Impression of Severity
CGIS	Clinician Global Impression of Severity
CIOMS	Council for International Organizations of Medical Sciences
C_{max}	maximum observed plasma concentration
COA	Clinical Outcome Assessment
CONSORT	Consolidated Standards of Reporting Trials
CRF	case report form
CRP	C-reactive protein
CTCAE	Common Terminology Criteria for Adverse Events
DBL	database lock
DFRF	Directed Follow-up Report Form
DMC	Data Monitoring Committee
DPARP	FDA Division of Pulmonary, Allergy, and Rheumatology Products
DRE	disease-related event
DRI	Dietary Reference Intake
DSM	Diagnostic and Statistical Manual of Mental Disorders
ECG	electrocardiogram

Abbreviation	Definition
EC/IRB	Ethics Committee/Institutional Review Board
eCRF	electronic Case Report Form
EDC	electronic data capture
ET	Early Termination
EU	European Union
FDA	Food and Drug Administration
FSH	follicle stimulating hormone
GCP	good clinical practice
GPP	good publication practice
HAE	hypersensitivity adverse event
HIPAA	Health Insurance Portability and Accountability Act
HPA	hyperphenylalaninemia
HRT	hormonal replacement therapy
HRV	hypersensitivity reaction visit
ICF	Informed Consent Form
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
ICMJE	International Committee of Medical Journal Editors
IgE	immunoglobulin E
IgG	immunoglobulin G
IgM	immunoglobulin M
IMP	investigational medical products
IND	investigational new drug
IP	investigational product
IQ	intelligence quotient
ISR	injection site reaction
I/T/M	Induction/Titration/Maintenance (dosing regimen)
LS	least square
MedDRA	Medical Dictionary for Regulatory Activities
MNT	medical nutritional therapy
NAb	neutralizing antibody
NCI-CTCAE	National Cancer Institute – Common Terminology Criteria for Adverse Events
NIAID/FAAN	National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network
NIMP	non-investigational medical products
NPKUA	National PKU Alliance

Abbreviation	Definition
NSAID	nonsteroidal anti-inflammatory drug
PAH	phenylalanine hydroxylase
PAL	phenylalanine ammonia lyase
PDCO	European Medicines Agency's Paediatric Committee
PEG	polyethylene glycol
PFS	pre-filled syringe
PGIC	Patient Global Impression of Change
PGIS	Patient Global Impression of Severity
Phe	phenylalanine
PIP	Paediatric Investigational Plan
PK	pharmacokinetics
PKU	phenylketonuria
PKU-SSIS	PKU Symptom Severity and Impacts Scale
PRN	pro re nata (as needed)
rAvPAL-PEG	PEGylated recombinant Anabaena variabilis phenylalanine ammonia lyase
RCT	randomized control trial
RDI	recommended daily intake
REMS	Risk Evaluation and Mitigation Strategy
SAE	serious adverse event
SAP	Statistical Analysis Plan
sBLA	supplemental Biologics License Application
SC	Study Completion
SD	standard deviation
SoA	Schedule of Activities
SOI	Statement of Investigator
TAb	total anti-drug antibody
Tyr	tyrosine
ULN	upper limit of normal
US	United States
WOCBP	Woman of Childbearing Potential

10.8 Appendix 8: Protocol Amendment History

The Protocol Amendment Summary of Changes Table for the current amendment is located directly before the Table of Contents (TOC). This is the second amendment to this protocol.

Amendment 1 (26 January 2022):

Overall Rationale for the Amendment

The protocol is being amended in response to feedback received from the Food and Drug Administration (FDA) in a request for advice/information letter dated 2 December 2021, and in response to feedback received from the European Medicines Agency's Paediatric Committee (PDCO) in a Paediatric Investigational Plan (PIP) modification positive opinion. Additionally, minor typographic and consistency errors are also being corrected.

Section Number and Name	Description of Change	Brief Rationale
Synopsis Figure 1.2.2 (Dosing Schematic) 4.1 (Overall Design) 5.5 (Study Stopping Criteria) 6.5 (Dose Modification) Table 6.5.1 (Study Drug Dose Titration) 7.1 (Discontinuation of Study Treatment)	Clarified that pegvaliase should be discontinued in participants who have not achieved an adequate response (< 600 µmol/L) after 16 weeks of continuous treatment at 60 mg/day	Response to FDA's advice/information request letter dated 02 December 02 2021, per US Prescribing Information.
Synopsis 5.1 (Inclusion Criteria)	Inclusion criterion wording amended to reflect that patients should have failed both sapropterin dihydrochloride and Phe-restricted diet, not one "and/or" the other.	Response to FDA's advice/information request letter dated 02 December 02 2021
Synopsis 5.1 (Inclusion Criteria) 5.3.1 (Meals and Dietary Restrictions) 8.1.2 (Total Dietary Protein Intake, Dietary Monitoring, and Dietary Protocol Restrictions)	Added "…Inclusion criterion to include the willingness for patients to maintain stable diet under supervision of study dietician or adequately trained designee per investigator during study participation"	As per agreement with PDCO, reflected in the PIP modification approval letter (EMEA-001951-PIP01-16-M02)
Synopsis Table 3.1 (Objectives and Endpoints)	Additional exploratory endpoint added with regards to the comparison of participants' optimal maintenance pegvaliase dose across different age groups within the study	As per agreement with PDCO, reflected in the PIP modification approval letter (EMEA-001951-PIP01-16-M02)

Section Number and Name	Description of Change	Brief Rationale
Synopsis Table 1.3.1 (SoA Part 1, Pegvaliase Arm) Table 1.3.2 (SoA Part 2, Pegvaliase Arm) Table 1.3.5 (SoA Part 2, Diet-Only Arm) 4.1 (Overall Design) 4.2 (Scientific Rationale for Study Design) 4.3 (Justification of Dose and Dose Regimen) 6.5 (Dose Modifications)	Clarified dosing administration to occur in a hospital setting for the first 8 weeks and for all subsequent dose escalations.	As per agreement with PDCO, reflected in the PIP modification approval letter (EMEA-001951-PIP01-16-M02)
Synopsis 2.2 (Background) 4.1 (Overall Design) 4.2 (Scientific Rationale for Study Design) 5.1 (Inclusion Criteria)	Added the abbreviation “US” and the eligibility age criterion (12 to 15 years (EU), inclusive) in EU	Clarify the eligibility age criterion of the patient populations for EU and US given the pegvaliase approval in US for 18 years and older and in EU for 16 years and older
5.4 (Screen Failures)	Added clarifying language around participants who are rescreened.	Makes it clear which rescreened participants must performed all Screening Visits procedures and assessments when re-screened.
8.4 (Pharmacokinetics) Table 1.3.1 (SoA Part 1, Pegvaliase Arm) Table 1.3.2 (SoA Part 2, Pegvaliase Arm) Table 1.3.5 (SoA Part 2, Diet-Only Arm)	Added “A pre-dose sample should be collected within 2 hours of dosing”	PK window for pre-dose is now specified to ensure consistency in the timing of the PK samples for all participants
Synopsis 9.4.2 (Primary Endpoints)	Stratification blood Phe measurement will include 2 blood Phe measurement performed during Screening/Run-In only	The Day 1 pre-dose blood Phe sample for stratification was included in error and has been removed. The two blood Phe samples performed during Screening/Run-In are adequate for treatment stratification.
Table 1.3.1 (SoA Part 1, Pegvaliase Arm) Table 1.3.4 (SoA Part 1, Diet-Only Arm)	Removed “and subject appraisal of metabolic control” from footnotes.	Text had been included in error
Table 1.3.4 (SoA Part 1, Diet-Only Arm)	Tryptase measurement added at Week 73	Was omitted in error

Section Number and Name	Description of Change	Brief Rationale
Table 1.3.1 (SoA Part 1, Pegvaliase Arm) Table 1.3.4 (SoA Part 1, Diet-Only Arm)	Column title changed from Study Day -28/1 to Study Day -28/-1	Correcting error of missing minus (–) sign
Table 1.3.1 (SoA Part 1, Pegvaliase Arm) Table 1.3.5 (SoA Part 2, Diet-Only Arm)	Urine pregnancy test added at HRV	For consistency with Table 1.3.2
Table 1.3.1 (SoA Part 1, Pegvaliase Arm)	Complement C3/C4 measurements added at Week 13 and Week 21	For alignment with Table 1.3.4 for Week 85 and Week 96

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Signature Page for VV-CLIN-022462 v2.0

Every: Approval Task	PI	

Signature Page for VV-CLIN-022462 v2.0

CLINICAL STUDY PROTOCOL (EX-US [UNITED STATES]) 165-306
AMENDMENT 1 06JUN2022

Protocol Title:	A Phase 3 Multi-Center Study to Evaluate the Safety and Efficacy of Subcutaneous Injections of Pegvaliase in Adolescent Subjects (Ages 12-17) with Phenylketonuria Featuring an Open-Label Randomized Two-Arm (Active vs Diet-Only Control) Design
Protocol Number:	165-306
Amendment Number:	Amendment 1 (ex-US [United States])
Compound:	pegvaliase (formerly BMN 165)
Short Title:	A Phase 3 Multi-Center Study to Evaluate the Safety and Efficacy of Subcutaneous Injections of Pegvaliase in Adolescent Subjects (Ages 12-17) with Phenylketonuria
Study Phase:	Phase 3
Sponsor Name:	BioMarin Pharmaceutical Inc.
Legal Registered Address:	105 Digital Drive Novato, CA 94949
Regulatory Agency Identifier Number(s):	IND: 076269 EudraCT: 2021-005058-27
Amendment 1 Date:	26 January 2022
Amendment 1 Date (ex-US):	06 June 2022

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May not be divulged, published, or otherwise disclosed to others without prior written approval from BioMarin.
This study will be conducted according to the principles of Good Clinical Practice as described in the U.S. Code of Federal Regulations and the International Conference on Harmonisation Guidelines, including the archiving of essential documents.

I have read this study protocol and agree to conduct this study in compliance with the protocol, all applicable regulations, guidelines, and other information supplied to me.

Investigator Signature Date

Printed name: _____

Accepted f

PI

PI

PI

PI

or Signature PI Date

PI

Printed name: _____

PI

Medical monitor contact information is available in App ction 10.3.

PROTOCOL AMENDMENT SUMMARY OF CHANGES TABLE

DOCUMENT HISTORY	
Document	**Approval date**
Amendment 1 (ex-US [United States])	06 June 2022
Amendment 1	26 January 2022
Original Protocol	22 September 2021

Amendment 1 (ex-US [United States]) (06 June 2022):

Overall Rationale for the Amendment

The protocol has been amended following German Regulatory Authority (BfArM) feedback and for consistency with the Summary of Product Characteristics (SmPC); patients with a history of a severe systemic hypersensitivity reaction or a history of recurrent acute systemic hypersensitivity reactions to a PEGylated medicinal product will be excluded from study participation.

Section Number and Name	Description of Change	Brief Rationale
Section 1.1, Synopsis, and Section 5.2, Exclusion Criteria	Added criterion to exclude patients with a history of a severe systemic hypersensitivity reactions or a history of recurrent acute systemic hypersensitivity reactions to a PEGylated medicinal product from study participation.	The exclusion criterion has been added following German Regulatory Authority (BfArM) feedback and for consistency with the SmPC.

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1 PROTOCOL SUMMARY

1.1 Synopsis

Protocol Title: A Phase 3 Multi-Center Study to Evaluate the Safety and Efficacy of Subcutaneous Injections of Pegvaliase in Adolescent Subjects (Ages 12-17) with Phenylketonuria Featuring an Open-Label Randomized Two-Arm (Active vs Diet-Only Control) Design

Short Title: A Phase 3 Multi-Center Study to Evaluate the Safety and Efficacy of Subcutaneous Injections of Pegvaliase in Adolescent Subjects (Ages 12-17) with Phenylketonuria

Study Design Rationale:

This Phase 3 multicenter study is designed to evaluate the safety and efficacy of pegvaliase administered daily to adolescent subjects (ages 12 to 17 years old (US), inclusive, and 12 to 15 years old (ex-US [United States]), inclusive) with phenylketonuria (PKU). Subjects will be randomized in a 2:1 ratio to the active (pegvaliase) and control (diet-only) treatment arms, respectively, with 36 subjects receiving pegvaliase and 18 subjects managing their PKU with diet alone. The primary efficacy endpoint is the change from baseline in blood phenylalanine (Phe) following 72 w eeks on study (the end of Part 1). Beginning Week 73, subjects in the active (pegvaliase) treatment arm will continue to receive pegvaliase in the Extension Phase (Part 2) for up to an additional 80 weeks (through Week 153). Subjects in the control (diet only) treatment arm will initiate pegvaliase treatment beginning Week 73 and, from Weeks 73 through 145, follow the same dosing and assessment schedule that the active subjects followed from Weeks 1 through 73, after which they will receive pegvaliase for up to 8 additional weeks (through Week 153).

A randomized control trial (RCT) design, with diet-only management of PKU as the control, will allow for robust data collection and minimize the risk of confounding factors influencing the results. An open-label diet-only control arm is the best comparator for this age group as daily placebo injections would place undue burden on adolescent PKU patients.

In Part 1 (through the Week 73 visit), randomized subjects in both the control (diet-only) and active (pegvaliase) treatment arms will be required to maintain the same level of protein intake they established during Screening/Run-in. In Part 2, subjects in the control (diet-only) arm must continue to maintain this prescribed level of protein intake, whereas subjects randomized to the active (pegvaliase) arm whose blood Phe concentration is ≤ 360 µmol/L for a minimum of 4 weeks may adjust dietary protein intake based on individual subject

response to pegvaliase and guidance from the investigator. Subjects are required to maintain a consistent level of protein intake during the study to ensure that the safety and efficacy endpoints are attributable to pegvaliase rather than to changes in dietary protein intake.

Pegvaliase will be subcutaneously injected in a hospital setting for the first 8 weeks and thereafter at the clinic or at home by a trained subject, caregiver, adult observer, or visiting healthcare provider. Pegvaliase will also be injected in a hospital setting for any subsequent dose escalations. Administration at home makes daily treatment less burdensome for subjects, thereby improving treatment compliance.

An adult observer is required for a minimum of 1 hour post injection throughout the study. An adult observer is defined as someone who will be present with the subject during and after pegvaliase administration, is able to recognize the signs and symptoms of an anaphylaxis episode, and is able to call for emergency medical support and administer epinephrine, if warranted. The requirement for an adult observer is included to ensure that subjects self-administering pegvaliase would have support in recognizing and quickly responding to signs and symptoms of anaphylaxis.

The primary efficacy endpoint is the change from treatment naïve baseline in blood Phe following 72 weeks on study (the end of Part 1). Blood Phe level is the established clinical marker used to diagnose PKU and assess disease control, and reduction of blood Phe concentration to target levels is the universally acknowledged treatment goal for PKU patients.

Objectives and Endpoints:

The primary, secondary, and exploratory objectives and associated endpoints are shown below. Refer to the Schedule of Activities (SoA) for the complete listing of assessment timepoints.

Objectives	Endpoints
Primary	
To evaluate the efficacy of pegvaliase in adolescent subjects with PKU	The primary efficacy endpoint is change in blood Phe concentration from treatment naïve baseline following 72 weeks on study. Change in blood Phe from baseline is compared between subjects in the active (pegvaliase) and the control (diet-only) treatment arms.
To evaluate the safety of pegvaliase in adolescent subjects with PKU	• Adverse events (AEs), including serious AEs (SAEs) • Clinical laboratory tests (chemistry, hematology, and urinalysis) results • Vital signs • Physical examination (including height, weight) • Electrocardiogram (ECG) test results • Immunogenicity test results (anti-PEG IgG, anti-PEG IgM, anti-PAL IgG, anti-PAL IgM, Tab, Nab, and anti-pegvaliase IgE [hypersensitivity reaction visits only]).
Secondary	
To characterize total dietary protein intake in adolescent subjects with PKU after pegvaliase treatment	Total dietary protein intake must remain stable for change in blood Phe, the primary endpoint, to be accurately assessed. To gauge the stability of total protein intake, the change from baseline at Week 73 in total dietary protein intake (defined as the sum of the protein intake from medical food and intact food) will be measured.
To characterize the pharmacokinetics (PK) of pegvaliase in adolescent subjects with PKU	Trough plasma PK samples will be collected and analyzed as specified in the SoA. PK parameters assessed with intensive PK sampling will include $AUC_{0\text{-}24hr,ss}$, $C_{max,ss}$, $T_{max,ss}$, $t_{1/2}$, $CL_{ss/F}$, and $V_{dz/F}$. For subjects randomized to the active (pegvaliase) treatment arm, intensive PK sampling will be performed at Week 73 and for dose escalations to 20, 40, and 60 mg/day. For subjects randomized to the control (diet-only) treatment arm, there are no PK assessments in Part 1. In Part 2, intensive PK sampling will be performed at Week 145 and for dose escalations to 20, 40, and 60 mg/day.

Objectives	Endpoints
Exploratory Objectives	
To evaluate the effect of pegvaliase treatment on neurocognitive outcomes in adolescent subjects with PKU.	• The ADHD Rating Scale IV inattention subscale (ADHD-RS IV) rated by the parent/guardian and performed at Baseline (Day 1, pre-dose) and every 12 weeks throughout the study. • The Behavior Rating Inventory of Executive Function (BRIEF) rated by the parent/guardian and performed at Baseline (Day 1, pre-dose) and every 12 weeks throughout the study.
To compare subjects' optimal maintenance pegvaliase dose across different age groups within the study	• Time to subjects' optimal maintenance dose compared to older age subsets • Number of subjects who require the highest (60 mg/day) maintenance dose • Percentage/number of subjects in the blood Phe target ranges of < 600 µmol/L and < 360 µmol/L.
Explore the biochemical, molecular, and cellular aspects of PKU.	Blood will be collected to evaluate biochemical, molecular, and cellular aspects of PKU and to develop the assays used for these evaluations.
PAH genotyping	Testing will be performed on samples collected Day 1 pre-dose where allowed by local health authorities and the EC/IRB.

Overall Design:

Study 165-306 is an open-label, two-arm, randomized control evaluation of pegvaliase in approximately 54 subjects (ages 12 to 17 years old (US), inclusive, and 12 to 15 years old (ex-US), inclusive) with diet only as the control arm. To be eligible for the study, potential subjects must meet all eligibility requirements described in the protocol, including the following selected key inclusion criteria:

- Is 12 to 17 years old (US), inclusive, or 12 to 15 years old (ex-US), inclusive, at the start of the Screening/Runin Period (Day 28).
- Diagnosis of PKU and failure to maintain recommended blood Phe levels on existing management (sapropterin dihydrochloride and Phe-restricted diet) demonstrated by 2 blood Phe concentration measurements > 600 µmol/L during the Screening/Runin Period (7 to 10 days between blood Phe assessments) and average blood Phe concentration > 600 µmol/L over the past 12 months (per available data).
- Willing and able to maintain and adjust dietary and medical protein food intake according to the study protocol and under the supervision of study dietician or adequately trained designee per investigator during study participation.

- An adult (≥ 18 years of age) has been identified who is willing and competent to observe the subject during study drug administration and for a minimum of 1 hour following administration. A home healthcare nurse may perform the study drug observations consistent with local regulatory and site requirements.

Selected key exclusion criteria include the following:

- Have a history of a severe systemic hypersensitivity reaction or recurrence of a mild to moderate acute systemic hypersensitivity reaction to any of the excipients or another PEGylated medicinal product.
- Potential subjects are not eligible if they are unable to identify and/or communicate to others that they are experiencing symptoms of potential anaphylaxis due to cognitive impairment or other reasons.

Following enrollment, subjects will be randomized 2:1 to the active (pegvaliase) and control (diet-only) treatment arms, with 36 subjects receiving daily pegvaliase and 18 subjects managing PKU by diet alone. Treatment assignment will be stratified by Screening/Run-In blood Phe (mean of the 2 pre-treatment blood Phe assessments performed during the Screening/Run-in period) of ≤ 1000 µmol/L or > 1000 µmol/L. The primary efficacy endpoint is the change from baseline blood Phe following 72 weeks on study (the end of Part 1). Subjects in the active and control arms will follow the same visit schedules and perform the same assessments throughout the 72-week Primary Treatment Phase (Part 1) except that the control subjects will not be receiving pegvaliase so will not have PK draws or immunogenicity assessments.

After providing informed consent, subjects will enter the Screening/Run-in period, which will last 4 weeks and include 2 assessments of blood Phe levels, 7 to 10 days apart, to determine eligibility. Following confirmation that the 2 blood Phe results are > 600 µmol/L and all other eligibility criteria have been met, subjects may be enrolled in the study on Day 1. During the Screening/Run-in and the Primary Treatment Phase (Part 1), subjects will be required to maintain stable protein intake from both medical food and/or intact food.

The duration of Part 1 is 72 weeks, during which pegvaliase treatment will be initiated for subjects in the active arm using an Induction/Titration/Maintenance (I/T/M) dosing regimen. The I/T/M dosing regimen consists of Induction/Titration Dosing (9 weeks) and Maintenance Dosing. Required hospital setting visits during Part 1 will occur for all dosing visits for the first 8 weeks, every 4 weeks thereafter, and when the pegvaliase dose is escalated to 20, 40, or 60 mg/day. The assessments performed at the clinic visits include measurement of blood Phe, neurocognitive assessments, PK blood draws (active subjects only), and safety assessments.

Beginning Week 73, subjects in the active treatment arm will continue to receive pegvaliase in the Extension Phase (Part 2) for up to 80 additional weeks (through Week 153). Beginning Week 73, subjects in the control treatment arm will initiate pegvaliase treatment and follow a parallel schedule for dosing, study visits, and assessments in Weeks 73 through 145 to that followed by the active arm subjects during the Primary Treatment Phase (Part 1) from Weeks 1 through 73, after which they will receive pegvaliase for up to 8 additional weeks (through Week 153).

Brief Summary:

The purpose of Study 165-306 is to evaluate the safety and efficacy of pegvaliase and characterize the PK of pegvaliase in adolescent subjects with PKU. Study details include:

Condition/Disease: PKU is a rare autosomal recessive genetic disorder caused by mutations in the phenylalanine hydroxylase (PAH) gene, leading to an absence or deficiency in PAH enzyme activity and subsequent elevation of the amino acid phenylalanine (Phe) in the blood, known as hyperphenylalaninemia (HPA). Deficiency of PAH results in an abnormally elevated concentration of Phe, which is toxic to the brain. High Phe levels during infancy and early childhood cause profound neurocognitive and developmental defects, and poorly controlled blood Phe levels in older children and adults are associated with learning disabilities, attention deficit hyperactivity disorder, behavioral problems, and psychiatric symptoms.

Study Hypothesis: The study hypothesis is that subjects receiving pegvaliase will show a greater reduction in blood Phe following 72 weeks on study compared to subjects in the diet-only control group.

Study Duration: The study duration is up to 160 weeks for subjects randomized to active (pegvaliase) treatment. This includes a 4-week Screening/Run-in period, up to 152 weeks of pegvaliase treatment in Parts 1 and 2, and the Study Completion/Early Termination (SC/ET) visit 4 weeks after the final dose of pegvaliase. The study duration is also 160 weeks for subjects randomized to control (diet-only) treatment. This includes a 4-week Screening/Run-in period, 72 weeks managing PKU with diet alone while following the Part 1 assessment schedule, 72 weeks of pegvaliase treatment following the same Part 1 assessment schedule, 8 additional weeks of pegvaliase treatment, and the SC/ET visit 4 weeks after the final dose of pegvaliase.

Treatment Duration: The pegvaliase treatment duration will be 152 weeks for subjects randomized to the active (pegvaliase) treatment arm (n=36). The pegvaliase treatment

duration will be 80 weeks for subjects randomized to the control (diet-only) treatment arm (n=18).

Health Measurement/Observation: Reduction in blood Phe.

Visit Frequency: For both active and control subjects, hospital setting visits in Part 1 will occur for all dosing visits for the first 8 weeks, then every 4 weeks through Week 73 and for all dose escalations. For subjects randomized to active (pegvaliase) treatment, clinic visits in Part 2 will occur every 4 weeks through Week 153 and for all dose escalations. For subjects randomized to control (diet-only) treatment, hospital setting visits in Part 2 will occur for all dosing visits for the first 8 weeks, then every 4 weeks through Week 153. For subjects randomized to active (pegvaliase) treatment in Part 1 and subjects randomized to control (diet-only) treatment in Part 2, hospital setting visits will also occur when their dose level is escalated to 20, 40, and 60 mg/day.

Number of Subjects:

Approximately 54 subjects will be enrolled and randomized to the pegvaliase or diet-only groups in a 2:1 ratio. Assuming the mean (SD) reduction in Phe following 72 weeks on study is 640 (570) µmol/L in the pegvaliase group and 100 (250) µmol/L in the diet-only group, a total sample size of 54 subjects will provide more than 90% power to detect a treatment difference, based on the two-sample T-test with unequal variance and a significance level of 0.05 (two sided). The assumption of treatment effect is based on clinical data from previous pegvaliase studies and a PKU registry study. See the Statistical Analysis Plan (SAP) for additional details on the sample size calculation.

Approximately 72 subjects will be screened for study participation such that approximately 54 subjects will be enrolled in the study.

Note: Potential subjects who are screened for the purpose of determining eligibility for the study, but do not participate in the study, are not considered enrolled.

Treatment Groups and Duration:

The duration of the Primary Treatment Phase (Part 1) is 72 weeks, during which pegvaliase treatment will be initiated for subjects randomized to the active (pegvaliase) treatment arm using an I/T/M dosing regimen. Induction will consist of a 4-week period when subjects randomized to active drug will receive pegvaliase subcutaneously with a pre-filled syringe (PFS) at a fixed dose of 2.5 mg/week and during which the dosing regimen will not be modified in response to blood Phe concentration. Once subjects complete induction they will titrate up to a dose of 10 mg/day. The dosing frequency will be increased gradually during

titration dosing to daily (7 days/week). During Maintenance Dosing the dose will be increased to 20 mg/day, 40 mg/day, and 60 mg/day depending upon individual blood Phe lowering efficacy (per the investigator's discretion). The dose should be increased to 40 mg/day if blood Phe is > 360 µmol/L after 24 weeks dosing at 20 mg/day and increased to 60 mg/day if blood Phe is > 360 µmol/L after 16 weeks dosing at 40 mg/day. If a subject's blood Phe is > 600 µmol/L after 16 weeks of continuous treatment with pegvaliase at 60 mg/day, pegvaliase should be permanently discontinued. BioMarin recommends that the target blood Phe levels should be based on individual needs, with a target blood Phe ≤ 600 µmol/L or lower. During the Primary Treatment Phase (Part 1), subjects randomized to the control (diet-only) treatment arm will have their PKU managed by diet alone.

Following completion of Part 1, subjects randomized to active (pegvaliase) treatment will enter the Extension Phase (Part 2), during which subjects will continue dosing with pegvaliase at their optimal maintenance dose level for up to an additional 80 weeks (through Week 153) in order to evaluate the long-term safety and efficacy of pegvaliase in adolescent subjects with PKU. Following completion of Part 1, subjects randomized to control (diet-only) treatment will repeat the Part 1 assessment schedule while initiating pegvaliase dosing from Weeks 73 through 145, after which they will receive pegvaliase for up to 8 additional weeks (through Week 153).

Data Monitoring Committee:

An independent Data Monitoring Committee (DMC) will monitor the safety of study subjects. The DMC is an independent committee that acts in an advisory capacity to BioMarin.

1.2 Study Schema

Figure 1.2.1: Active (Pegvaliase) vs Control (Diet-Only) Treatment Arms (Randomized Treatment Assignment)

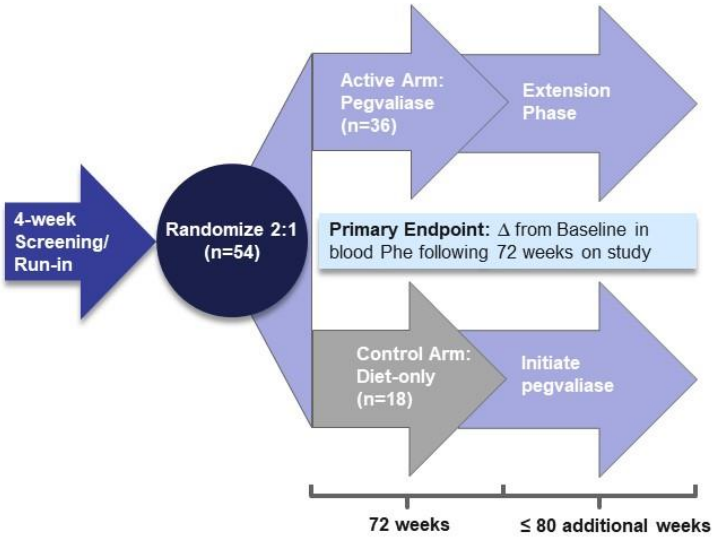
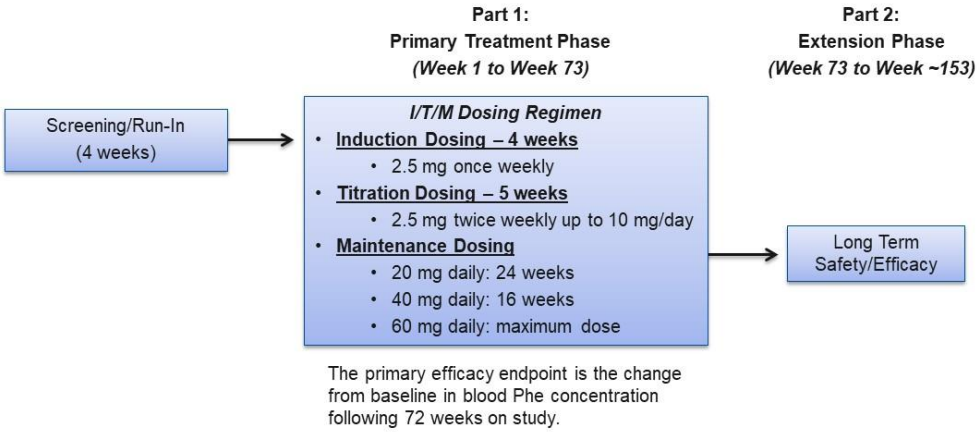


Figure 1.2.2: Dosing Schematic for Active (Pegvaliase) Treatment Arm



Note: The 18 control (diet-only) subjects will follow the Part 1 assessment schedule (except for pegvaliase dosing and PK/immunogenicity assessments) from Weeks 1 through Week 73 and then repeat the Part 1 assessment schedule including pegvaliase dosing and PK/immunogenicity assessments from Week 73 through 145, after which they will receive pegvaliase for 8 additional weeks (through Week 153).

Prior to dose escalation, the minimum amount of time pegvaliase can be dosed at 20 mg/day before escalating is 24 weeks and the minimum amount of time at 40 mg/day before escalating is 16 weeks. The dose should be increased to 40 mg/day if blood Phe is > 360 µmol/L after 24 weeks dosing at 20 mg/day, and increased to 60 mg/day if blood Phe is > 360 µmol/L after 16 weeks dosing at 40 mg/day. If a subject's blood Phe is > 600 µmol/L after 16 weeks of continuous treatment with pegvaliase at 60 mg/day, pegvaliase should be permanently discontinued.

1.3 Schedule of Activities (SoA)

Table 1.3.1: Schedule of Activities for Part 1 (Subjects Randomized to Pegvaliase Treatment Arm)

Assessment [a]	Study Week	Screen[b]	1	2	3	4	5	6	7	8	9	13	17	21	25	29	33	37	41	45	49	53	57	61	65	69	73		
	Study Day	-28/-1	1	8	15	22	29	36	43	50	57	85	113	141	169	197	225	253	281	309	337	365	393	421	449	477	505	HRV[c]	SC/ET[d]
	Window (Days)			±3	±3	±3	±3	±3	±3	±3	±3	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7		
Informed consent		X																											
Drug administration training [e]			X																										
Study drug observer training [f]		X																											
Demographics		X																											
Medical history [g]		X																											
HIV, hepatitis B and C screens [h]		X																											
PAH genotyping [i]			X																										
12 lead ECG		X	X																										X
Physical examination [j]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Weight		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Height		X	X				X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
Vital signs		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Clinical safety laboratory tests [k]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
C-reactive protein		X	X								X		X		X		X		X		X		X		X		X	X	X
Erythrocyte sedimentation rate [l]		X	X																										X
Serum cortisol [m]			X												X						X						X		X
Complements C3 and C4			X				X				X	X	X	X	X		X		X		X		X		X		X	X	X
Tryptase			X																									X	
Urine albumin/creatinine ratio [n]			X								X		X		X		X		X		X		X		X		X	X	X
Urine pregnancy test [o]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
3-day diet diary [p]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
PK (plasma pegvaliase) [q]			X				X				X	X	X	X	X		X		X		X		X		X		X		X

	Study Week	Screen[b]	1	2	3	4	5	6	7	8	9	13	17	21	25	29	33	37	41	45	49	53	57	61	65	69	73		
	Study Day	-28/-1	1	8	15	22	29	36	43	50	57	85	113	141	169	197	225	253	281	309	337	365	393	421	449	477	505	HRV[c]	SC/ET[d]
Assessment [a]	Window (Days)			±3	±3	±3	±3	±3	±3	±3	±3	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7		
Blood for exploratory analyses [i]			X				X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
Blood Phe, tyrosine [r]		X [b]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
Immunogenicity assessments [s]			X	X	X	X	X	X	X	X	X	X	X	X	X		X		X		X		X		X		X	X	X
ADHD-RS IV [t]			X									X			X			X			X			X			X		X
BRIEF [u]			X									X			X			X			X			X			X		X
Weekly call (non-visit weeks) [v]																													
Adverse events [w]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant medication [w]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Administer study drug [x]			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		

ACTH, adrenocorticotropic hormone; ADHD-RS IV, attention deficit hyperactivity disorder rating scale IV; BRIEF, Behavior Rating Inventory of Executive Function; EC/IRB, Ethics Committee/Institutional Review Board; ECG, electrocardiogram; eCRF, electronic Case Report Form; HRV, hypersensitivity reaction visit; NSAID, non-steroidal anti-inflammatory drug; PAH, phenylalanine hydroxylase; PE, physical examination; PK, pharmacokinetics; PKU, phenylketonuria; SC/ET, Study Completion/Early Termination visit.

[a] Scheduled visits take place at the hospital setting (for all dosing visits for the first 8 weeks, and for all dose escalation visits), clinic, or, with the approval of the investigator or designee, virtually. For virtual visits the investigator/designee performs assessments while online with the subject, apart from specimen collections and vital signs, which are performed by home healthcare personnel. Non-clinic visits are via telephone. Assessments are performed pre-dose unless otherwise specified.

[b] After written informed consent, screening assessments must be performed within 28 days prior to Part 1, Day 1. Subjects will be assessed for blood Phe concentration during Screening/Run-in with 2 measurements 7 to 10 days apart. These 2 blood Phe measurements should be scheduled to occur: (1) in the Day -25 to Day -23 window; and (2) 7 to 10 days later (ie, in the Day -18 to Day -13 window).

[c] If the investigator determines that the NCI-CTCAE Grade $\geq$ 3 hypersensitivity reaction is related to administration with study drug, the subject will be asked to return to the clinic within 24 hours of event onset for a hypersensitivity reaction visit (HRV). Sampling for anti-pegvaliase IgE should be performed 8 to 24 hours after event onset and before the next dose of study drug.

[d] Subjects should perform the Study Completion (SC) Visit 4 weeks after the last dose of study drug or after completion of study participation, whichever occurs last. For subjects who terminate from the study early, the Early Termination (ET) Visit occurs within 4 weeks of the last study drug dose or after withdrawal from study participation, whichever occurs last. Subjects who discontinue from study drug early will be asked to continue to complete the remaining study assessments until study completion as long as such continued participation will not detrimentally affect the health, safety, and welfare of the subject per investigator determination.

[e] Once subject/caregiver competence is documented, the subject/caregiver administers study drug daily.

[f] Must be completed prior to administration of the first dose of study drug. Additional observers identified after screening must be trained.

[g] PKU history includes highest blood Phe (and age).

[h] Subjects with documented negative results within the last 4 weeks do not need to be retested.

[i] Sample collection should be performed where allowed by country-specific regulations and EC/IRB.

[j] A full physical examination (PE) will be performed at Screening, Baseline, Week 73, and the SC/ET visit. A brief PE will be performed at other visits. A full PE will include, at a minimum, general appearance (head, eyes, ears, nose, and throat) and an assessment of cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, musculoskeletal, and neurologic systems. A brief PE may be performed at the discretion of the investigator based on the subject's clinical condition and may include, but is not limited to, general appearance and an assessment of cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, musculoskeletal, and neurologic systems.

[k] It is recommended that urine samples are obtained as a first or second morning void. In the event of elevated urinary protein on a test result, a repeat urinalysis should be performed. This repeat urine sample must be performed in the morning at the first or second morning void to allow for accurate test results and may be performed by a home healthcare nurse.

[l] To be done by a local laboratory.

[m] Serum cortisol samples should be taken before study drug administration in the morning before 10:00 am. A home healthcare nurse may collect samples for subject convenience. If 2 results are low and abnormal, the subject will be asked to perform additional sampling for plasma ACTH and a low-dose conventional ACTH stimulation test or the ACTH test method based on the clinical practice at the site.

[n] It is recommended that urine samples be obtained as a first or second morning void. Subjects with a confirmed urine/albumin creatinine ratio of ≥ 100 mg/g should be referred to a nephrologist for consultation if results were within normal range at Baseline. Subjects who had elevated results at Baseline followed by a confirmed subsequent increase of 100-200 mg/g from Baseline should also be referred to a nephrologist for consultation.

[o] To be done locally (if applicable). If urine pregnancy test is positive or equivocal, serum pregnancy test (central laboratory) must be done.

[p] Subjects should record at home all food, beverages, special low-protein foods, and medical foods consumed during the 3 consecutive days preceding a study visit. No change in protein (dietary and medical food) intake is allowed in Part 1. During Screening/Run-in, 3-day diet diary assessments are performed 3 times, each time covering the 3-day periods prior to blood Phe assessments (and collected on the day of those assessments). The 3 Screening/Run-in blood Phe assessments are scheduled to occur (1) at Day -25 to Day -23; (2) 7 to 10 days later (ie, in the Day -18 to Day -13 window); and (3) at Day 1 pre-dose. Thus, the 3 day diet assessments will assess diet for (1) the 3 days within the Day -28 to Day -23 window immediately prior to the first blood Phe assessment; (2) the 3 days within the Day -21 to Day -15 window immediately prior to the second blood Phe assessment; and (3) for Day -3 to Day 1.

[q] Blood is collected before study drug administration (pre-dose) within 2 hours of dosing. Intensive PK sampling will be performed at Week 73 and at dose escalation visits in all subjects. Samples will be taken at pre-dose (within 2 hours of dosing), 2, 4, 8, 12, and 24 hours post dose. The 24-hour sample will be taken prior to the next daily dose.

[r] Blood is collected for blood Phe analysis after fasting a minimum of 2.5 hours. In addition to the blood draws for blood Phe analysis collected in the clinic and shown in the SoA, there are 5 required blood collections that may be drawn either at the clinic or elsewhere (eg, at home). These are to be collected at Weeks 11, 15, and 19, and 2 weeks prior to the planned escalations to 40 and 60 mg/day.

[s] Immunogenicity assays include total anti-pegvaliase antibodies (TAb), anti-PAL IgG, anti-PAL IgM, anti-PEG IgM, anti-PEG IgG, and neutralizing antibodies (NAb). At the HRV, only anti-pegvaliase IgE will be assessed.

[t] Completed by parent/guardian.

[u] Completed by parent/guardian.

[v] Between scheduled clinic visits, the clinic staff will contact the subject weekly to monitor if the subject is experiencing problems with drug administration, to ask about any AEs or concomitant medications, and to answer questions.

[w] AEs and concomitant medications should be noted whenever a subject is assessed by study personnel. Following signed informed consent and prior to the first dose of pegvaliase, only SAEs associated with study procedures are collected. After the first dose all AEs and SAEs are collected until 4 weeks after the last study drug dose or the Early Termination Visit, whichever occurs last. If there is a severe (Grade 3) injection site reaction, the subject may be referred to a dermatologist for consultation and a skin biopsy (optional; if clinically indicated). If photographs of the severe injection site reaction are taken, they may be collected by the sponsor.

[x] Subjects must be premedicated with an H1 antagonist, and an H2 antagonist, and an antipyretic approximately 2 to 3 hours prior to each dose of study drug until they reach the Maintenance Phase, defined as when subjects achieve blood Phe ≤ 600 µmol/L while they are on a stable dose of pegvaliase. Premedication may be considered during the Maintenance Phase at the investigator's discretion, eg, approximately 2 to 3 hours prior to study drug for 1 week upon reintroduction of study drug, upon resolution of an AE, following any dose interruption of ≥ 4 days, and for any dose increases in the Extension Phase. Subjects may also be premedicated at any other time in the study per investigator discretion. When an NSAID is administered as a premedication, it should be given with food. For non-clinic visits, subjects are asked about study drug administration.

Table 1.3.2: Schedule of Activities for Part 2 (Subjects Randomized to Pegvaliase Treatment Arm)

Assessment [a]	Study Week	73[b]	77	81	85	89	93	97	101	105	109	113	117	121	125	129	133	137	141	145	149	153		
	Study Day	505	533	561	589	617	645	673	701	729	757	785	813	841	869	897	925	953	981	1009	1037	1065	HRV[c]	SC/ET[d]
	Window (Days)	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7		
Study drug observer training [e]																								
12 lead ECG																								X
Physical examination [f]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Weight	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Height	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
Vital signs	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Clinical safety laboratory tests [g]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
C-reactive protein	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Erythrocyte sedimentation rate [h]																								X
Serum cortisol [i]	X						X						X								X			X
Complements C3 and C4	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Tryptase																							X	
Urine albumin/creatinine ratio [j]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Urine pregnancy test [k]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
3-day diet diary [l]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			X
PK (plasma pegvaliase) [m]	X		X		X		X		X		X		X		X		X		X		X			X
Blood for exploratory analyses [n]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			X
Blood Phe, tyrosine [o]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Immunogenicity assessments [p]	X		X		X		X		X		X		X		X		X		X		X	X	X	X
ADHD-RS IV [q]	X			X			X			X			X			X			X					X
BRIEF [r]	X			X			X			X			X			X			X					X
Weekly call (non-visit weeks) [s]																								
Adverse events [t]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant medication [t]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

Assessment [a]	Study Week	73[b]	77	81	85	89	93	97	101	105	109	113	117	121	125	129	133	137	141	145	149	153		
	Study Day	505	533	561	589	617	645	673	701	729	757	785	813	841	869	897	925	953	981	1009	1037	1065	HRV[c]	SC/ET[d]
	Window (Days)	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7		
Administer study drug [u]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		

ACTH, adrenocorticotropic hormone; ADHD-RS IV, attention deficit hyperactivity disorder rating scale IV; BRIEF, Behavior Rating Inventory of Executive Function; ECG, electrocardiogram; eCRF, electronic Case Report Form; HRV, hypersensitivity reaction visit; PE, physical examination; PK, pharmacokinetics; PKU, phenylketonuria; NSAID, non-steroidal anti-inflammatory drug; SC/ET, Study Completion/Early Termination visit.

[a] Scheduled visits take place at the hospital setting (for all dose escalation visits), clinic, or, with the approval of the investigator or designee, virtually. For virtual visits the investigator/designee performs assessments while online with the subject, apart from specimen collections and vital signs, which are performed by home healthcare personnel. Non-clinic visits are via telephone. Assessments are performed pre-dose unless otherwise specified.

[b] The Week 73 visit marks both the end of the Primary Treatment Phase (Part 1) and the beginning of the Extension Phase (Part 2). The pre-dose assessments constituting the end of the Primary Treatment Phase and the administration of study drug constituting the beginning of the Extension Phase are shown in the Week 73 columns of both Table 1.3.1 and Table 1.3.2.

[c] If the investigator determines that the NCI-CTCAE Grade ≥ 3 hypersensitivity reaction is related to administration with study drug, the subject will be asked to return to the clinic within 24 hours of event onset for a hypersensitivity reaction visit (HRV). Sampling for anti-pegvaliase IgE should be performed 8 to 24 hours after event onset and before the next dose of study drug.

[d] Subjects should perform the Study Completion (SC) Visit 4 weeks after the last dose of study drug or after completion of study participation, whichever occurs last. For subjects who terminate from the study early, the Early Termination (ET) Visit occurs within 4 weeks of the last study drug dose or after withdrawal from study participation, whichever occurs last. Subjects who discontinue from study drug early will be asked to continue to complete the remaining study assessments until study completion as long as such continued participation will not detrimentally affect the health, safety, and welfare of the subject per investigator determination.

[e] Additional observers identified after screening must be trained.

[f] A full PE will be performed at Week 73 and the SC/ET visit. A brief PE will be performed at other visits. A full PE will include, at a minimum, general appearance (head, eyes, ears, nose, and throat) and an assessment of cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, musculoskeletal, and neurologic systems. A brief PE may be performed at the discretion of the investigator based on the subject's clinical condition and may include, but is not limited to, general appearance and an assessment of cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, musculoskeletal, and neurologic systems.

[g] It is recommended that urine samples are obtained as a first or second morning void. In the event of elevated urinary protein on a test result, a repeat urinalysis should be performed. This repeat urine sample must be performed in the morning at the first or second morning void to allow for accurate test results and may be performed by a home healthcare nurse.

[h] To be done by a local laboratory.

[i] Serum cortisol samples should be taken before study drug administration in the morning before 10:00 am. A home healthcare nurse may collect samples for subject convenience. If 2 results are low and abnormal, the subject will be asked to perform additional sampling for plasma ACTH and a low-dose conventional ACTH stimulation test or the ACTH test method based on the clinical practice at the site.

[j] It is recommended that urine samples be obtained as a first or second morning void. Subjects with a confirmed urine/albumin creatinine ratio of ≥ 100 mg/g should be referred to a nephrologist for consultation if results were within normal range at Baseline. Subjects who had elevated results at Baseline followed by a confirmed subsequent increase of 100-200 mg/g from Baseline should also be referred to a nephrologist for consultation.

[k] To be done locally (if applicable). If urine pregnancy test is positive or equivocal, serum pregnancy test (central laboratory) must be done.

[l] Subjects should record at home all food, beverages, special low-protein foods, and medical foods consumed during the 3 consecutive days preceding a study visit. No change in protein (dietary and medical food) intake is allowed in Part 1.

[m] Blood is collected before study drug administration (pre-dose) within 2 hours of dosing. Intensive PK sampling will be performed at Week 73 and at dose escalation visits in all subjects. Samples will be taken at pre-dose (within 2 hours of dosing), 2, 4, 8, 12, and 24 hours post dose. The 24-hour sample will be taken prior to the next daily dose.

[n] Sample collection should be performed where allowed by country-specific regulations and EC/IRB.

[o] Blood is collected for blood Phe analysis after fasting a minimum of 2.5 hours.

[p] Immunogenicity assays include total anti-pegvaliase antibodies (TAb), anti-PAL IgG, anti-PAL IgM, anti-PEG IgM, anti-PEG IgG, and neutralizing antibodies (NAb). At the HRV, only anti-pegvaliase IgE will be assessed.

[q] Completed by parent/guardian.

[r] Completed by parent/guardian.

[s] Between scheduled clinic visits, the clinic staff will contact the subject weekly to monitor if the subject is experiencing problems with drug administration, to ask about any AEs or concomitant medications, and to answer questions.

[t] AEs and concomitant medications should be noted whenever a subject is assessed by study personnel. All AEs and SAEs are collected until 4 weeks after the last study drug dose or the Study Completion Visit/Early Termination visit, whichever occurs last. If there is a severe (Grade 3) injection site reaction, the subject may be referred to a dermatologist for consultation and a skin biopsy (optional; if clinically indicated). If photographs of the severe injection site reaction are taken, they may be collected by the sponsor.

[u] Subjects must be premedicated with an H1 antagonist, and an H2 antagonist, and an antipyretic approximately 2 to 3 hours prior to each dose of study drug until they reach the Maintenance Phase, defined as when subjects achieve blood Phe ≤ 600 µmol/L while they are on a stable dose of pegvaliase. Premedication may be considered during the Maintenance Phase at the investigator's discretion, eg, approximately 2 to 3 hours prior to study drug for 1 week upon reintroduction of study drug, upon resolution of an AE, following any dose interruption of ≥ 4 days, and for any dose increases in the Extension Phase. Subjects may also be premedicated at any other time in the study per investigator discretion. When an NSAID is administered as a premedication, it should be given with food. For non-clinic visits, subjects are asked about study drug administration.

Table 1.3.3: Schedule of Activities for Part 1 (Subjects Randomized to Diet-Only Treatment Arm)

Assessment [a]	Study Week	Screen[b]	1	2	3	4	5	6	7	8	9	13	17	21	25	29	33	37	41	45	49	53	57	61	65	69	73	
	Study Day	-28/-1	1	8	15	22	29	36	43	50	57	85	113	141	169	197	225	253	281	309	337	365	393	421	449	477	505	SC/ET[c]
	Window (Days)			±3	±3	±3	±3	±3	±3	±3	±3	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	
Informed consent		X																										
Drug administration training [d]																											X	
Study drug observer training [e]																											X	
Demographics		X																										
Medical history (allergy, PKU) [f]		X																										
HIV, hepatitis B and C screens [g]		X																										
PAH genotyping [h]			X																									
12 lead ECG		X	X																								X	X
Physical examination [i]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Weight		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Height		X	X				X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital signs		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Clinical safety laboratory tests [j]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
C-reactive protein		X	X								X		X		X		X		X		X		X		X		X	X
Erythrocyte sedimentation rate [k]		X	X																								X	X
Serum cortisol [l]			X												X						X						X	X
Complements C3 and C4			X				X				X		X		X		X		X		X		X		X		X	X
Tryptase			X																								X	
Urine albumin/creatinine ratio [m]			X								X		X		X		X		X		X		X		X		X	X
Urine pregnancy test [n]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
3-day diet diary [o]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Blood for exploratory analyses [h]			X				X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Blood Phe, tyrosine [p]		X [b]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
ADHD-RS IV [q]			X									X			X			X			X			X			X	X

	Study Week	Screen[b]	1	2	3	4	5	6	7	8	9	13	17	21	25	29	33	37	41	45	49	53	57	61	65	69	73	
	Study Day	-28/-1	1	8	15	22	29	36	43	50	57	85	113	141	169	197	225	253	281	309	337	365	393	421	449	477	505	SC/ET[c]
Assessment [a]	Window (Days)			±3	±3	±3	±3	±3	±3	±3	±3	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	
BRIEF [r]			X									X			X			X			X			X			X	X
Weekly call (non-visit weeks) [s]																												
Adverse events [t]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant medication [t]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

ACTH, adrenocorticotropic hormone; ADHD-RS IV, attention deficit hyperactivity disorder rating scale IV; BRIEF, Behavior Rating Inventory of Executive Function; EC/IRB, Ethics Committee/Institutional Review Board; ECG, electrocardiogram; eCRF, electronic Case Report Form; HRV, hypersensitivity reaction visit; PAH, phenylalanine hydroxylase; PE, physical examination; PK, pharmacokinetics; PKU, phenylketonuria; SC/ET, Study Completion/Early Termination visit.

[a] Scheduled visits take place at the clinic or, with the approval of the investigator or designee, virtually. For virtual visits the investigator/designee performs assessments while online with the subject, apart from specimen collections and vital signs, which are performed by home healthcare personnel. Non-clinic visits are via telephone.

[b] After written informed consent, screening assessments must be performed within 28 days prior to Part 1, Day 1. Subjects will be assessed for blood Phe concentration during Screening/Run-in with 2 measurements 7 to 10 days apart. These 2 blood Phe measurements should be scheduled to occur: (1) in the Day -25 to Day -23 window; and (2) 7 to 10 days later (ie, in the Day -18 to Day -13 window).

[c] Subjects should perform the Study Completion (SC) Visit 4 weeks after the last dose of study drug or after completion of study participation, whichever occurs last. For subjects who terminate from the study early, the Early Termination (ET) Visit occurs within 4 weeks of the last study drug dose or after withdrawal from study participation, whichever occurs last. Subjects who discontinue from study drug early will be asked to continue to complete the remaining study assessments until study completion as long as such continued participation will not detrimentally affect the health, safety, and welfare of the subject per investigator determination.

[d] Once subject/caregiver competence is documented, the subject/caregiver administers study drug daily.

[e] Must be completed prior to administration of the first dose of study drug in Part 2. Additional observers identified after Screening must be trained.

[f] PKU history includes highest blood Phe (and age).

[g] Subjects with documented negative results within the last 4 weeks do not need to be retested.

[h] Sample collection should be performed where allowed by country-specific regulations and EC/IRB.

[i] A full PE will be performed at Screening, Baseline, Week 73, and the SC/ET visit. A brief PE will be performed at other visits. A full PE will include, at a minimum, general appearance (head, eyes, ears, nose, and throat), and an assessment of cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, musculoskeletal, and neurologic systems. A brief PE may be performed at the discretion of the investigator based on the subject's clinical condition and may include, but is not limited to, general appearance and an assessment of cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, musculoskeletal, and neurologic systems.

[j] It is recommended that urine samples are obtained as a first or second morning void. In the event of elevated urinary protein on a test result, a repeat urinalysis should be performed. This repeat urine sample must be performed in the morning at the first or second morning void to allow for accurate test results and may be performed by a home healthcare nurse.

[k] To be done by a local laboratory.

[l] Serum cortisol samples should be taken before study drug administration in the morning before 10:00 am. A home healthcare nurse may collect samples for subject convenience. If 2 results are low and abnormal, the subject will be asked to perform additional sampling for plasma ACTH and a low-dose conventional ACTH stimulation test or the ACTH test method based on the clinical practice at the site.

[m] It is recommended that urine samples be obtained as a first or second morning void. Subjects with a confirmed urine/albumin creatinine ratio of ≥ 100 mg/g should be referred to a nephrologist for consultation if results were within normal range at Baseline. Subjects who had elevated results at Baseline followed by a confirmed subsequent increase of 100-200 mg/g from Baseline should also be referred to a nephrologist for consultation.

[n] To be done locally (if applicable). If urine pregnancy test is positive or equivocal, serum pregnancy test (central laboratory) must be done.

[o] Subjects should record at home all food, beverages, special low-protein foods, and medical foods consumed during the 3 consecutive days preceding a study visit. No change in protein (dietary and medical food) intake is allowed in Part 1. During Screening/Run-in, 3-day diet diary assessments are performed 3 times, each time covering the 3-day periods prior to blood Phe assessments (and collected on the day of those assessments). The 3 Screening/Run-in blood Phe assessments are scheduled to occur (1) at Day -25 to Day -23; (2) 7 to 10 days later (ie, in the Day -18 to Day -13 window); and (3) at Day 1 pre-dose. Thus, the 3 day diet assessments will assess diet for (1) the 3 days within the Day -28 to Day -23 window immediately prior to the first blood Phe assessment; (2) the 3 days within the Day -21 to Day -15 window immediately prior to the second blood Phe assessment; and (3) for Day -3 to Day 1.

[p] Blood is collected for blood Phe analysis after fasting a minimum of 2.5 hours. In addition to the blood draws for blood Phe analysis collected in the clinic and shown in the SoA, there are 3 required blood collections that may be drawn either at the clinic or elsewhere (eg, at home). These are to be collected at Weeks 11, 15, and 19.

[q] Completed by parent/guardian.

[r] Completed by parent/guardian.

[s] Between scheduled clinic visits, the clinic staff will contact the subject weekly to ask about any AEs or concomitant medications, and to answer questions.

[t] AEs and concomitant medications should be noted whenever a subject is assessed by study personnel. Following signed informed consent all AEs and SAEs are collected throughout Part 1 or until the Early Termination Visit, whichever occurs last.

Table 1.3.4: Schedule of Activities for Part 2 (Pegvaliase Treatment for Subjects Randomized to Diet-Only Treatment Arm)

	Study Week	73[b]	74	75	76	77	78	79	80	81	85	89	93	97	101	105	109	113	117	121	125	129	133	137	141	145	149	153		
	Study Day	505	512	519	526	533	540	547	554	561	589	617	645	673	701	729	757	785	813	841	869	897	925	953	981	1009	1037	1065	HRV[c]	SC/ET[d]
Assessment [a]	Window (Days)	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7		
Drug administration training [e]		X																												
Study drug observer training [f]		X																												
12 lead ECG		X																												X
Physical examination [g]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Weight		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Height		X				X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
Vital signs		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Clinical safety laboratory tests [h]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
C-reactive protein		X								X		X		X		X		X		X		X		X		X		X	X	X
Erythrocyte sedimentation rate [i]		X																												X
Serum cortisol [j]		X												X						X						X				X
Complements C3 and C4		X				X				X	X	X	X	X		X		X		X		X		X		X		X	X	X
Tryptase		X																											X	
Urine albumin/creatinine ratio [k]		X								X		X		X		X		X		X		X		X		X		X	X	X
Urine pregnancy test [l]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
3-day diet diary [m]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
PK (plasma pegvaliase) [n]		X				X				X	X	X	X	X		X		X		X		X		X		X		X		X
Blood for exploratory analyses [o]		X				X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
Blood Phe, tyrosine [p]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
Immunogenicity assessments [q]		X	X	X	X	X	X	X	X	X	X	X	X	X		X		X		X		X		X		X		X	X	X
ADHD-RS IV [r]		X									X			X			X			X			X			X				X
BRIEF [s]		X									X			X			X			X			X			X				X
Weekly call (non-visit weeks) [t]																														
Adverse events [u]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

Assessment [a]	Study Week	73[b]	74	75	76	77	78	79	80	81	85	89	93	97	101	105	109	113	117	121	125	129	133	137	141	145	149	153		
	Study Day	505	512	519	526	533	540	547	554	561	589	617	645	673	701	729	757	785	813	841	869	897	925	953	981	1009	1037	1065	HRV[c]	SC/ET[d]
	Window (Days)	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7		
Concomitant medication [u]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Administer study drug [v]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		

ACTH, adrenocorticotropic hormone; ADHD-RS IV, attention deficit hyperactivity disorder rating scale IV; BRIEF, Behavior Rating Inventory of Executive Function; ECG, electrocardiogram; eCRF, electronic Case Report Form; HRV, hypersensitivity reaction visit; NSAID, non-steroidal anti-inflammatory drug; PE, physical examination; PK, pharmacokinetics; PKU, phenylketonuria; SC/ET, Study Completion/Early Termination visit.

[a] Scheduled visits take place at the hospital setting (for all dosing visits for the first 8 weeks, and for all dose escalation visits), clinic, or, with the approval of the investigator or designee, virtually. For virtual visits the investigator/designee performs assessments while online with the subject, apart from specimen collections and vital signs, which are performed by home healthcare personnel. Non-clinic visits are via telephone. Assessments are performed pre-dose unless otherwise specified.

[b] For Cohort B control subjects, the Week 73 visit marks both the end of Part 1, when their PKU is treated with diet alone, and the beginning of Part 2, when they receive pegvaliase. The Week 73 pre-dose assessments constituting the end of Part 1 are shown in the Week 73 column of Table 1.3.3. These same assessments, used as baseline assessments for Part 2, are shown in the Week 73 column of Table 1.3.4, along with the administration of study drug that marks the beginning of Part 2.

[c] If the investigator determines that the NCI-CTCAE Grade ≥ 3 hypersensitivity reaction is related to administration with study drug, the subject will be asked to return to the clinic within 24 hours of event onset for a hypersensitivity reaction visit (HRV). Sampling for anti-pegvaliase IgE should be performed 8 to 24 hours after event onset and before the next dose of study drug.

[d] Subjects should perform the Study Completion (SC) Visit 4 weeks after the last dose of study drug or after completion of study participation, whichever occurs last. For subjects who terminate from the study early, the Early Termination (ET) Visit occurs within 4 weeks of the last study drug dose or after withdrawal from study participation, whichever occurs last. Subjects who discontinue from study drug early will be asked to continue to complete the remaining study assessments until study completion as long as such continued participation will not detrimentally affect the health, safety, and welfare of the subject per investigator determination.

[e] Once subject/caregiver competence is documented, the subject/caregiver administers study drug daily.

[f] Must be completed prior to administration of the first dose of study drug. Additional observers identified after screening must be trained.

[g] A full PE will be performed at Week 73, Week 145, and the SC/ET visit. A brief PE will be performed at other visits. A full physical examination will include, at a minimum, general appearance (head, eyes, ears, nose, and throat), and an assessment of cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, musculoskeletal, and neurologic systems. A brief physical examination may be performed at the discretion of the investigator based on the subject's clinical condition and may include, but is not limited to, general appearance and an assessment of cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, musculoskeletal, and neurologic systems.

[h] It is recommended that urine samples are obtained as a first or second morning void. In the event of elevated urinary protein on a test result, a repeat urinalysis should be performed. This repeat urine sample must be performed in the morning at the first or second morning void to allow for accurate test results and may be performed by a home healthcare nurse.

[i] To be done by a local laboratory.

[j] Serum cortisol samples should be taken before study drug administration in the morning before 10:00 am. A home healthcare nurse may collect samples for subject convenience. If 2 results are low and abnormal, the subject will be asked to perform additional sampling for plasma ACTH and a low-dose conventional ACTH stimulation test or the ACTH test method based on the clinical practice at the site.

[k] It is recommended that urine samples be obtained as a first or second morning void. Subjects with a confirmed urine/albumin creatinine ratio of ≥ 100 mg/g should be referred to a nephrologist for consultation if results were within normal range at Baseline. Subjects who had elevated results at Baseline followed by a confirmed subsequent increase of 100-200 mg/g from Baseline should also be referred to a nephrologist for consultation.

[l] To be done locally (if applicable). If urine pregnancy test is positive or equivocal, serum pregnancy test (central laboratory) must be done.

[m] Subjects should record at home all food, beverages, special low-protein foods, and medical foods consumed during the 3 consecutive days preceding a study visit. No change in protein (dietary and medical food) intake is allowed in Part 1.

[n] Blood is collected before study drug administration (pre-dose) within 2 hours of dosing. Intensive PK sampling will be performed at Week 145 and at dose escalation visits in all subjects. Samples will be taken at pre-dose (within 2 hours of dosing), 2, 4, 8, 12, and 24 hours post dose. The 24-hour sample will be taken prior to the next daily dose.

[o] Sample collection should be performed where allowed by country-specific regulations and EC/IRB.

[p] Blood is collected for blood Phe analysis after fasting a minimum of 2.5 hours. In addition to the blood draws for blood Phe analysis collected in the clinic and shown in the SoA, there are 5 required blood collections that may be drawn either at the clinic or elsewhere (eg, at home). These are to occur at Weeks 83, 87, and 91, and 2 weeks prior to the planned escalations to 40 and 60 mg/day.

[q] Immunogenicity assays include total anti-pegvaliase antibodies (TAb), anti-PAL IgG, anti-PAL IgM, anti-PEG IgM, anti-PEG IgG, and neutralizing antibodies (NAb). At the HRV, only anti-pegvaliase IgE will be assessed.

[r] Completed by parent/guardian.

[s] Completed by parent/guardian.

[t] Between scheduled clinic visits, the clinic staff will contact the subject weekly to monitor if the subject is experiencing problems with drug administration, to ask about any AEs or concomitant medications, and to answer questions.

[u] AEs and concomitant medications should be noted whenever a subject is assessed by study personnel. Beginning with start of study treatment, All AEs and SAEs are collected until 4 weeks after the last study drug dose or the Study Completion Visit/Early Termination visit, whichever occurs last. If there is a severe (Grade 3) injection site reaction, the subject may be referred to a dermatologist for consultation and a skin biopsy (optional; if clinically indicated). If photographs of the severe injection site reaction are taken, they may be collected by the sponsor.

[v] Subjects must be premedicated with an H1 antagonist, and an H2 antagonist, and an antipyretic approximately 2 to 3 hours prior to each dose of study drug until they reach the Maintenance Phase, defined as when subjects achieve blood Phe ≤ 600 µmol/L while they are on a stable dose of pegvaliase. Premedication may be considered during the Maintenance Phase at the investigator's discretion, eg, approximately 2 to 3 hours prior to study drug for 1 week upon reintroduction of study drug, upon resolution of an AE, following any dose interruption of ≥ 4 days, and for any dose increases in the Extension Phase. Subjects may also be premedicated at any other time in the study per investigator discretion. When an NSAID is administered as a premedication, it should be given with food. For non-clinic visits, subjects are asked about study drug administration.

2 INTRODUCTION

Phenylketonuria (PKU, OMIM 261600) is a rare autosomal recessive genetic disorder that is characterized by an inability of the body to break down the amino acid, phenylalanine (Phe), and is caused by mutations in the gene encoding phenylalanine hydroxylase (PAH). PAH catalyzes the conversion of the essential amino acid Phe to tyrosine, and this enzymatic activity is facilitated by tetrahydrobiopterin (BH4). PAH deficiency results in abnormally elevated concentrations of Phe. The estimated incidence of PKU is 1 in 15,000 births in the US (NIHCDP 2001).

The pathophysiology of PKU is well understood. A substantial body of evidence confirms that elevated Phe is directly toxic to cells in the brain, where it causes inhibition of protein synthesis in humans and animals (Wall 1990), affects the normal morphology of myelinating proteins (Dyer 1996), and leads to arrested or delayed development of dendrites and synapses in the cerebral cortex (Huttenlocher 2000). Neuropathology in PKU has been shown to derive from oxidative stress, impaired neurotransmitter metabolism, impaired protein synthesis and lipid metabolism, and brain energy metabolism alterations (Schuck 2015). The neurotoxicity of elevated Phe is a direct effect of Phe itself (Kaufman 1989). No abnormalities in Phe metabolites are observed in patients with PKU and normal Phe metabolites are toxic only at concentrations that are much higher than those reported in individuals with PKU.

With newborn screening followed by Medical Nutritional Therapy (MNT), the most severe manifestation of the disease, mental retardation, is prevented. Phe restriction with MNT involves consumption of semisynthetic medical foods that provide a source of Phe-free protein, modified low-protein foods, a small amount of natural protein (to provide required amounts of Phe), and other supplements (eg, vitamins and minerals) to correct nutritional deficiencies that can result from Phe restriction. Without highly restricted Phe intake and MNT, individuals with PKU quickly reach and sustain toxic concentrations of blood Phe. Individuals with classic PKU may have a complete absence or profound deficiency of PAH and typically show very high elevations of blood Phe (≥ 1200 µmol/L).

Historically, it was recommended that adherence to the Phe-restricted diet could end after age 6, but later studies have shown that patients who maintained Phe control longer had better outcomes in neuropsychological performance and areas of attention and speed of processing (Cerone 1999; Channon 2007; Rey 1996). A robust body of research suggests that early blood Phe control through dietary management is imperative in PKU populations, and that adult intelligence quotient (IQ) levels are correlated with the duration and vigilance of dietary management in childhood and adolescence (Pietz 1997; Smith 2000; Stemerdink 2000; Waisbren 1999).

To be optimally effective, a PKU diet needs to be started immediately after birth and continued for life. The American College of Medical Genetics and Genomics (ACMG) practice guidelines for PKU recommend life-long management and maintenance of metabolic control as essential to optimal functioning of individuals with PKU, with a goal of maintaining blood Phe concentrations ≤ 360 µmol/L (Vockley 2014). Similar European guidelines for the management of PKU recommend treatment target Phe levels of ≤ 600 µmol/L for patients older than 12 years (van Spronsen 2017).

Compliance with diet is a significant component of disease management, but life-long dietary compliance can be extremely difficult. A study conducted over 6 years at 4 treatment centers showed that dietary compliance in PKU populations was found to decline with age. Specifically, during infancy and in pre-school aged children, compliance with restrictive PKU diet is high because their diet is carefully managed by a parent and nutritionist. However, when PKU children start school, their blood Phe levels increase because of changes in socialization and changes in their palate, making compliance to a Phe-restricted diet more challenging (Walter 2002; Walter 2004). As children age, they become more likely to find formula and synthetic low-Phe foods distasteful and they become exposed to a broader range of food and peer pressure, both of which encourage noncompliance (MacDonald 2010). Adolescents/young adults with PKU are at particular risk for non-compliance with MNT as they transition away from childhood when parental oversight of dietary control decreases (Brown 2016). In addition, adolescents/young adults who have stopped MNT and attempt to resume a Phe-restricted diet into adulthood have difficulty maintaining the diet, with 31% of patients no longer maintaining dietary control after 10 months of restarting (Schuett 1985).

Therefore, despite the best efforts of the medical community and patients, life-long therapy with MNT with Phe restriction is not sustainable nor feasible as a treatment option for the majority of patients with PKU (Walter 2002; Burton 2010; Aoki 2007). The inability to adhere to MNT results in uncontrolled blood Phe levels associated with executive dysfunction, depression, and a variety of behavioral and psychiatric problems (Moyle 2007; Pietz 1997; Smith 2000; Waisbren 1999). Adolescents with PKU with poor metabolic control represent a patient population in great need of additional therapeutic options.

BioMarin has developed pegvaliase as a subcutaneous enzyme substitution treatment to reduce blood Phe levels in adult patients with PKU. Enzyme replacement therapy with PAH is not feasible due to the instability of PAH in plasma and the difficulty with efficiently delivering an intact, active form of the enzyme to the liver (Harding 2003; Kim 2004). Pegvaliase is a genetically modified phenylalanine ammonia lyase (PAL) enzyme product of

the cyanobacterium *Anabaena variabilis*, which is PEGylated to decrease immunogenicity and increase half-life. Unlike PAH, the PAL enzyme is active in plasma and, independently of cofactor BH4 which is required for PAH activity, catalyzes Phe to trans-cinnamic acid (t-CA) and ammonia, which are metabolized in the liver and excreted in the urine, respectively. The pegvaliase mechanism of action corrects the underlying PAH deficiency that leads to inability to metabolize Phe, increased Phe levels in the body, and consequent neurocognitive, neuropsychiatric, and executive functioning deficits.

A comprehensive review of pegvaliase is contained in the Investigator's Brochure (IB). Investigators must review this document prior to initiating this study.

2.1 Study Rationale

Although it is recommended that adolescents with PKU maintain good metabolic control as they move into adulthood (Vockley 2014), in separate surveys > 60% of adolescents (13 to 17 years of age) in the US have blood Phe concentrations above recommended targets (Jurecki 2017) and 79% of patients 15 to 19 years report blood Phe concentrations above targets (Walter 2002). Adolescents aged 12 to 17 years with PKU report difficulties with maintaining blood Phe control as they transition away from childhood when parental oversight of dietary and blood Phe control decreases (Brown 2016; van Spronsen 2017). In a 2015 National PKU Alliance (NPKUA) survey conducted among over 625 adults and families, approximately 25% of those caregivers indicated that their children's average blood Phe levels were in excess of the ACMG-recommended targeted range (120 to 360 µmol/L), and over half of all respondents (adults and caregivers) reported that it was somewhat to very difficult to manage their PKU (Brown 2016).

Adolescent patients report difficulties adhering to a Phe-restricted diet to maintain blood Phe levels within a safe range (Brown 2016; van Spronsen 2017), and a clinic survey assessing compliance with clinical guidance and dietary restriction showed that 60% of adolescents aged 13 to 17 were non-compliant, with an associated increase of Phe levels to > 360 µmol/L in 50% of patients and Phe levels > 600 µmol/L in 22% of patients. The same survey demonstrated that non-compliance starts as early as ages 5 to 12, and non-adherence to guidelines increases with age.

For adolescent patients at increased risk for loss of metabolic control maintained during childhood, a variety of adverse neuropsychological deficits (including deficits in executive functioning and psychiatric symptoms, such as anxiety, depression, and phobias) develop later in life when there is relaxation of blood Phe control (Huijbregts 2018; Diamond 1997). Adolescent PKU patients with blood Phe levels ≤360 µmol/L have been shown to perform better at neuropsychological and neurocognitive tasks (Huijbregts 2002; Jahja 2014).

The approval of Palynziq addressed a previous unmet medical need, providing adult PKU patients with a treatment that substantially reduced blood Phe concentrations and resulted in the stabilization of Phe above and beyond diet. The reduction and stabilization of blood Phe were associated with improvement in unmanaged symptoms, including neurocognitive symptoms. There is potential for improvement in these outcomes for adolescents as well given the demonstrated efficacy in adults. Therefore, expansion of Palynziq use to include adolescent patients 12 to 17 years with uncontrolled blood Phe concentrations greater than 600 µmol/L on existing management would broaden the patient population who can benefit from Palynziq treatment and address a currently underserved patient population.

The pathogenesis and pathophysiology of PKU, driven by an absence or deficiency in PAH enzyme leading to high Phe concentration, is applicable to both adolescents and adults. The pegvaliase mechanism of action to correct the underlying metabolic deficiency of PAH gene is the same in both adults and adolescents. Adolescent PKU patients are not expected to have specific ongoing developmental changes that could result in a different safety or efficacy outcome with pegvaliase treatment.

2.2 Background

Palynziq received marketing authorization in the US and European Union (EU). The original Biologics License Application (BLA) for Palynziq was approved by the Food and Drug Administration (FDA) at dosages up to 40 mg/day on 24 May 2018, to reduce blood Phe concentrations in adult patients with phenylketonuria (PKU) who have uncontrolled blood Phe concentrations greater than 600 µmol/L on existing management (BLA STN 761079). A supplemental Biologics License Application (sBLA) that included the addition of the maximum maintenance dose of 60 mg/day and long-term safety and efficacy data for adults was approved by the FDA on 06 October 2020. A Risk Evaluation and Mitigation Strategy (REMS) is a required element of the Palynziq approval in the US. Palynziq was approved by the European Commission on 03 May 2019 at dosages up to 60 mg/day to reduce blood Phe concentrations in patients aged 16 years and older who have inadequate blood Phe control (defined as blood Phe levels greater than 600 µmol/L) despite prior management with available treatment options.

BioMarin recognizes that PKU is a serious genetic disease diagnosed at birth that requires strict management of blood Phe levels from birth and continuing through life. Now that a positive benefit to risk profile has been established in adults, BioMarin is proposing this pegvaliase clinical study in adolescent PKU patients (ages 12 to 17 years old (US), inclusive, and 12 to 15 years old (ex-US), inclusive).

2.3 Benefit/Risk Assessment

Regulatory approval of Palynziq in the US and the EU was based on demonstration of efficacy from the Phase 2 and Phase 3 data of the pegvaliase clinical development program. The approval of pegvaliase addressed a previous unmet medical need, providing adult PKU patients with a treatment that substantially reduced blood Phe concentrations and resulted in the stabilization of Phe above and beyond diet. This reduction and stabilization of blood Phe was also associated with improvement in unmanaged symptoms including neurocognitive symptoms. Given the demonstrated efficacy in adults, there is the potential for improvement in these outcomes for adolescents.

The important identified risks for pegvaliase include acute systemic hypersensitivity reaction (equivalent to anaphylaxis for the purpose of this protocol; see Section 8.3.8.1 for how this risk is defined), angioedema, serum sickness, hypophenylalaninemia, persistent arthralgia, and severe injection site reaction (see Section 8.3.8 for event definitions). These events have been effectively treated and managed in the clinical trial and post-marketing settings. Healthcare providers, patients, and observers are trained in the recognition and management of both an acute systemic hypersensitivity reaction or angioedema, including the use of injectable epinephrine (adrenaline) and the need to seek immediate medical assistance. Premedication is required during Induction/Titration Dosing to help mitigate the risk of hypersensitivity reactions, including acute systemic hypersensitivity reactions. While hypophenylalaninemia has not been reported frequently in the post-marketing setting, it was observed during the clinical studies, where it was managed with decreases in pegvaliase dosing and increases in dietary protein intake. Subjects will undergo frequent blood Phe monitoring during induction and titration and throughout dosing and will be required to dose reduce with confirmed hypophenylalaninemia (ie, 2 consecutive blood Phe levels < 30 µmol/L).

The important potential risks of treatment with pegvaliase also include potential complications of immune complex formation resulting in end-organ damage, fetal developmental toxicity, and acute systemic hypersensitivity reaction in patients taking other concurrent injectables containing PEG. No evidence of immune complex-mediated end organ damage or any early clinical signs potentially related to immune complex mediated disease have been reported in the clinical trial or commercial setting. Concurrent injectables containing PEG are prohibited during study treatment with the exception of COVID vaccinations (see Section 6.8.1).

The potential risks are justified by the anticipated benefits that may be afforded to adolescent subjects. Each subject will have a comprehensive plan that includes frequent monitoring of

adverse events, blood Phe levels, and pregnancy during the study. Safety will be assessed by adverse event reporting and clinical laboratory assessments.

More detailed information about the known and expected benefits and risks and reasonably expected adverse events of pegvaliase may be found in the IB.

2.3.1 Risk Assessment

Table 2.3.1.1: Risks Associated with Study Drug

Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Acute systemic hypersensitivity reaction	In the completed Phase 2 and Phase 3 clinical trials, 16 out of 285 (6%) subjects experienced 25 episodes of acute systemic hypersensitivity reactions of any severity. Acute systemic hypersensitivity reactions were managed by administration of adrenaline (44%; 11/25 episodes), corticosteroids (56%; 14/25 episodes), antihistamines (56%; 14/25 episodes), and/or oxygen (8%; 2/25 episodes). Four out of 16 (1%; 4/285) subjects experienced a total of 5 episodes of an acute systemic hypersensitivity reaction that were considered severe. The underlying mechanism for acute systemic hypersensitivity reaction observed in clinical trials was non-IgE mediated Type III (immune-complex mediated) hypersensitivity. Acute systemic hypersensitivity reaction was most frequent during the Induction/Titration Phase (5% of subjects; 19 episodes over mean treatment duration of 13 months) and decreased in the Maintenance Phase (2% of subjects; 6 episodes over a mean treatment duration of 33 months). The risk of an acute systemic hypersensitivity reaction occurring is 6-fold higher in the Induction/Titration Phase compared to the Maintenance Phase.	To mitigate the risk of an acute systemic hypersensitivity reaction, an adult observer is required for a minimum of 1 hour post injection and premedication including H1 and H2 antagonists and an antipyretic is required 2 to 3 hours before each injection until subjects reach the Maintenance Phase.[a] Premedication may be considered during the Maintenance Phase at the investigator's discretion. Subjects will be provided with 2 epinephrine injectors and instructed to carry both injectors with them at all times. Subjects and adult observers will be trained to recognize the signs and symptoms of anaphylaxis and, if these reactions occur, to call for emergency medical support and administer an epinephrine injector. For the duration of the study, each subject will be contacted weekly by study site personnel to monitor for drug administration problems and adverse events (AEs). Risk mitigation measures also include dosing instructions for other hypersensitivity adverse events (Table 6.5.1.1).
Angioedema	In the completed Phase 2 and Phase 3 clinical trials, 21 out of 285 (7%) subjects experienced 37 episodes of mild to moderate episodes of angioedema (symptoms include one or more of the following: pharyngeal edema, swollen tongue, lip swelling, mouth swelling, eyelid edema and face edema) occurring independent of acute systemic	To mitigate the risk of hypersensitivity reactions, premedication including H1 and H2 antagonists and an antipyretic is required 2 to 3 hours before each injection until subjects reach the Maintenance Phase.[a] Premedication may be considered during the maintenance phase at the investigator's discretion.

Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
	hypersensitivity reaction. Angioedema was most frequent during the Induction/Titration Phase (6% of subjects; 27 episodes over mean treatment duration of 13 months) and decreased in the Maintenance Phase (3% of subjects; 10 episodes over mean treatment duration of 33 months). The risk of angioedema occurring is 4.5-fold higher in the Induction/Titration Phase compared to the Maintenance Phase.	An adult observer is required for a minimum of 1 hour post injection throughout the study. For the duration of the study, each subject will be contacted weekly by study site personnel to monitor for drug administration problems and AEs. Risk mitigation measures also include dosing instructions for hypersensitivity adverse events (Table 6.5.1.1).
Serum sickness	In the completed Phase 2 and Phase 3 clinical trials, serum sickness was reported in 7 out of 285 (2%) subjects. Serum sickness was most frequent during the Induction/Titration Phase (2% of subjects; 6 episodes over mean treatment duration of 13 months) and decreased in the Maintenance Phase (0.6% of subjects; 1 episode over mean treatment duration of 33 months). The risk of serum sickness occurring is greater than 2-fold higher in the Induction/Titration Phase compared to the Maintenance Phase.	To mitigate the risk of hypersensitivity reactions, premedication including H1 and H2 antagonists and an antipyretic is required 2 to 3 hours before each injection until subjects reach the Maintenance Phase of dosing.[a] Premedication may be considered during the Maintenance Phase at the investigator's discretion. An adult observer is required for a minimum of 1 hour post injection throughout the study. For the duration of the study, each subject will be contacted weekly by study site personnel to monitor for drug administration problems and AEs. Risk mitigation measures also include dosing instructions for hypersensitivity adverse events (Table 6.5.1.1).
Persistent arthralgia (> 6 month duration)	In the completed Phase 2 and Phase 3 clinical trials, 245 out of 285 (86%) subjects experienced episodes consistent with arthralgia (including back pain, musculoskeletal pain, pain in extremity, and neck pain). Arthralgia occurred as early as the first dose and can occur at any time during treatment. Arthralgia was most frequent during the Induction/Titration Phase (79% of subjects; 1269 episodes over mean treatment duration of 13 months) and decreased in the Maintenance Phase (67% of subjects; 673 episodes over mean treatment duration of 33 months). The risk of arthralgia occurring is 3.1-fold higher in the Induction/Titration Phase compared to the Maintenance Phase. Seven (7%) percent of the subjects experienced persistent arthralgia (≥ 6 months). The risk of persistent	To mitigate the risk of hypersensitivity reactions, premedication including H1 and H2 antagonists and an antipyretic is required 2 to 3 hours before each injection until subjects reach the Maintenance Phase of dosing.[a] Premedication may be considered during the Maintenance Phase at the investigator's discretion. An adult observer is required for a minimum of 1 hour post injection throughout the study. For the duration of the study, each subject will be contacted weekly by study site personnel to monitor for drug administration problems and AEs. Risk mitigation measures also include dosing instructions for hypersensitivity adverse events (Table 6.5.1.1).

Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
	arthralgia may have significant impact on quality of life. Severe arthralgia (severe pain limiting self-care activities of daily living) was experienced in 5% of subjects. Dose was not changed for 96% of episodes and all persistent arthralgia episodes resolved without sequelae.	
Severe injection site reaction	Injection site reaction (ISR) was reported in 266 out of 285 (93%) subjects. The most common ISR (occurring in at least 10% of subjects) was reaction, erythema, bruising, pruritus, pain, swelling, rash, induration, and urticaria. Injection site reaction was most frequent during the Induction/Titration Phase (90% of subjects; 3908 episodes over mean treatment duration of 13 months) and decreased in the Maintenance Phase (66% of subjects; 1226 episodes over mean treatment duration of 33 months). The risk of an ISR occurring is 5.2-fold higher in the Induction/Titration Phase compared to the Maintenance Phase. Three ISRs consistent with granulomatous skin lesions were reported (each reaction occurring in 1 patient): granulomatous dermatitis (occurred 15 months after pegvaliase treatment and lasted 16 days), xanthogranuloma (occurred 12 months after pegvaliase treatment and lasted 21 months), and necrobiosis lipoidica diabeticorum (occurred 9 months after pegvaliase treatment and lasted 9 months). Necrobiosis lipoidica diabeticorum was treated with steroid injections and was complicated by a *Pseudomonas* infection. All of these injection site reactions resolved.	To mitigate the risk of hypersensitivity reactions, premedication including H1 and H2 antagonists and an antipyretic is required 2 to 3 hours before each injection until subjects reach the Maintenance Phase of dosing.[a] Premedication may be considered during the Maintenance Phase at the investigator's discretion. An adult observer is required for a minimum of 1 hour post injection throughout the study. For the duration of the study, each subject will be contacted weekly by study site personnel to monitor for drug administration problems and AEs. Risk mitigation measures also include dosing instructions for hypersensitivity adverse events (Table 6.5.1.1). Any subject who experiences a severe (Grade 3) skin reaction will be asked to consult with a dermatologist for diagnosis and, if clinically indicated, a skin biopsy will be recommended. A Skin Reaction eCRF must be completed.
Hypophenylalaninemia	In the completed Phase 2 and Phase 3 clinical trials, 46% of the subjects developed hypophenylalaninemia (blood phenylalanine levels below 30 µmol/L on two consecutive measurements). The risk of hypophenylalaninemia occurring is 2.1-fold higher in the maintenance phase compared to the Induction/Titration phase. In the completed adult clinical studies, dose reductions were effective in	Blood Phe levels will be frequently monitored during induction and titration dosing and regularly monitored during maintenance dosing in both the Primary Treatment Phase and the Extension Phase of the study. Risk mitigation measures, including protocol-required adjustments in the pegvaliase dose and/or dietary intake of Phe, will be implemented to mitigate the potential risk of chronic

Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
	managing hypophenylalaninemia in subjects experiencing hypophenylalaninemia despite appropriate levels of protein intake.	hypophenylalaninemia levels (< 30 µmol/L) in adolescent subjects.
Complications of immune complex formation resulting in end-organ damage	No evidence from clinical trials or post-marketing reports of immune complex-mediated end organ damage or any early clinical signs potentially related to immune complex mediated disease.	Risk mitigation measures include monitoring clinical laboratory tests (chemistry, hematology, urinalysis) and adverse events for immune complex-mediated end-organ damage. These mitigations will also allow early detection of adverse effects of PEG accumulation. For the duration of the study, each subject will be contacted weekly by study site personnel to monitor for AEs.
Fetal development toxicity	There are limited data from the use of pegvaliase in pregnant women. Animal studies have shown maternal reproductive toxicity that was associated with decreased blood phenylalanine concentrations below normal levels. Uncontrolled blood phenylalanine levels (hyperphenylalaninemia) before and during pregnancy are associated with increased risk for miscarriage, major birth defects (including microcephaly and major cardiac malformations), intrauterine fetal growth retardation and future intellectual disability with low IQ. Additional risk to the unborn child due to hypophenylalaninemia is not established.	If sexually active, male or female subjects must not plan to become pregnant (self or partner) and must use 2 acceptable methods of contraception while participating in the study beginning at Screening and for 4 weeks after discontinuing study drug. Contraceptive use must be consistent with the guidance provided in Appendix 4, Section 10.4 of this protocol.
Unpredictable immune-mediated response in patients < 16 years	The adult development program was restricted to subjects aged > 16 years of age. Review of spontaneous adverse reaction reports in patients < 16 years of age, using Palynziq in the commercial setting has not identified any unpredictable immune responses. The adverse reactions reported in patients < 16 years of age are consistent with the known safety profile of Palynziq as presented in the USPI and SmPC.	To mitigate the risk of hypersensitivity reactions, premedication including H1 and H2 antagonists and an antipyretic is required 2 to 3 hours before each injection until subjects reach the Maintenance Phase. [a] Premedication may be considered during the Maintenance Phase at the investigator's discretion. An adult observer is required for a minimum of 1 hour post injection throughout the study. For the duration of the study, each subject will be contacted weekly by study site personnel to monitor for drug administration problems and AEs. Risk mitigation measures also include dosing instructions for hypersensitivity adverse events (Table 6.5.1.1).

Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Acute systemic hypersensitivity reactions in patients taking other concurrent injectables containing PEG	PEGylated proteins have the potential to elicit an immune response. Because antibodies bind to the PEG portion of pegvaliase, there may be potential for binding with other PEGylated therapeutics and increased hypersensitivity to other PEGylated injectables. In a single dose study of pegvaliase in adult subjects with PKU, two patients receiving concomitant injections of medroxyprogesterone acetate suspension containing PEG experienced hypersensitivity reactions.	Risk mitigation measures include prohibiting any injectable drugs containing polyethylene glycol (PEG; other than pegvaliase), including medroxyprogesterone injection, within 3 months prior to the start of Screening/Run-in and during study participation with the exception of COVID vaccinations (see Section 6.8.1).

[a] Subjects are in the Maintenance Phase when they achieve blood Phe ≤600 µmol/L while they are on a stable dose of pegvaliase. This is distinct from Maintenance Dosing, defined as the period following Induction/Titration Dosing when Maintenance Doses (20, 40, or 60 mg/day) are administered, as outlined in Table 6.5.1.

2.3.2 Benefit Assessment

The overall clinical benefit of pegvaliase was established in patients with PKU based on data from completed Phase 2 and Phase 3 clinical studies. The approval of Palynziq addressed a previous unmet medical need, providing adult PKU patients with a treatment that substantially reduced blood Phe concentrations and resulted in the stabilization of Phe above and beyond diet. This reduction and stabilization was also associated with improvement in unmanaged symptoms including neurocognitive symptoms. Given the demonstrated efficacy in adults, there is the potential for improvement in these outcomes for adolescents.

2.3.3 Overall Benefit/Risk Conclusion

Results from the robust pegvaliase clinical development program demonstrated that pegvaliase treatment up to 60 mg/day demonstrated a positive benefit-risk profile in patients with PKU with uncontrolled blood Phe levels > 600 µmol/L.

Taking into account the measures taken to minimize risk to subjects participating in this study, the anticipated benefits that may be afforded to adolescent PKU patients treated with pegvaliase outweigh the potential risks.

3 OBJECTIVES AND ENDPOINTS

The primary, secondary, and exploratory objectives and associated endpoints are shown in Table 3.1.

Table 3.1: Objectives and Endpoints

Objectives	Endpoints
Primary	
To evaluate the efficacy of pegvaliase in adolescent subjects with PKU	The primary efficacy endpoint is change in blood Phe concentration from treatment naïve baseline following 72 weeks on study. Change in blood Phe from baseline is compared between subjects in the active (pegvaliase) and the control (diet-only) treatment arms (see Section 9.4.2).
To evaluate the safety of pegvaliase in adolescent subjects with PKU	• Adverse events (AEs), including serious AEs (SAEs) • Clinical laboratory tests (chemistry, hematology, and urinalysis) results • Vital signs • Physical examination (including height, weight) • Electrocardiogram (ECG) test results • Immunogenicity test results (anti-PEG IgG, anti-PEG IgM, anti-PAL IgG, anti-PAL IgM, TAb, NAb, and anti-pegvaliase IgE [hypersensitivity reaction visits only]).
Secondary	
To characterize total dietary protein intake in adolescent subjects with PKU after pegvaliase treatment	Total dietary protein intake must remain stable for change in blood Phe, the primary endpoint, to be accurately assessed. To gauge the stability of total protein intake, the change from baseline at Week 73 in total dietary protein intake (defined as the sum of the protein intake from medical food and intact food) will be measured (see Section 9.4.3).
To characterize the pharmacokinetics (PK) of pegvaliase in adolescent subjects with PKU	Trough plasma PK samples will be collected and analyzed as specified in the SoA. PK parameters assessed with intensive PK sampling will include $AUC_{0\text{-}24hr,ss}$, $C_{max,ss}$, $T_{max,ss}$, $t_{1/2}$, $CL_{ss/F}$, and $V_{dz/F}$. For subjects randomized to the active (pegvaliase) treatment arm, intensive PK sampling will be performed at Week 73 and for dose escalations to 20, 40, and 60 mg/day. For subjects randomized to the control (diet-only) treatment arm, there are no PK assessments in Part 1. In Part 2, intensive PK sampling will be performed at Week 145 and for dose escalations to 20, 40, and 60 mg/day (see Section 8.4).

Objectives	Endpoints
Exploratory Objectives	
To evaluate the effect of pegvaliase treatment on neurocognitive outcomes in adolescent subjects with PKU.	• The ADHD Rating Scale IV inattention subscale (ADHD-RS IV) rated by the parent/guardian and performed at Baseline (Day 1, pre-dose) and every 12 weeks throughout the study (see Section 8.1.3.1.1). • The Behavior Rating Inventory of Executive Function (BRIEF) rated by the parent/guardian and performed at Baseline (Day 1, pre-dose) and every 12 weeks throughout the study (see Section 8.1.3.1.2).
To compare subjects' optimal maintenance pegvaliase dose across different age groups within the study	• Time to subjects' optimal maintenance dose compared to older age subsets • Number of subjects who require the highest (60 mg/day) maintenance dose • Percentage/number of subjects in the blood Phe target ranges of < 600 µmol/L and < 360 µmol/L.
Explore the biochemical, molecular, and cellular aspects of PKU.	Blood will be collected to evaluate biochemical, molecular, and cellular aspects of PKU and to develop the assays used for these evaluations.
PAH genotyping	Testing will be performed on samples collected Day 1 pre-dose where allowed by local health authorities and the EC/IRB.

4 STUDY DESIGN

4.1 Overall Design

This Phase 3 multi-center study is designed to evaluate the safety and efficacy of pegvaliase administered daily to adolescent subjects (ages 12 to 17 years old (US), inclusive, and 12 to 15 years old (ex-US), inclusive) with PKU. Pegvaliase will be subcutaneously injected in a hospital setting for the first 8 weeks and thereafter at the clinic or at home by a trained subject, caregiver, adult observer, or visiting healthcare provider. Pegvaliase will also be injected in a hospital setting for any subsequent dose escalations. The study design is summarized in Figure 1.2.1.

The study will enroll approximately 54 US and ex-US subjects (ages 12 to 17 years old (US), inclusive, and 12 to 15 years old (ex-US), inclusive) who will be evaluated using an open-label, 2-arm, randomized control design, with diet-only management of PKU as the control. Subjects will be randomized in a 2:1 ratio to the active and control arms, respectively, with 36 subjects receiving pegvaliase and 18 subjects managing their PKU with diet alone.

The primary efficacy endpoint is the change from baseline in blood Phe following 72 weeks on study (the end of Part 1). Beginning Week 73, subjects in the active treatment arm will continue to receive pegvaliase in the Extension Phase (Part 2) for up to an additional 80 weeks (through Week 153) in order to evaluate the long-term safety and efficacy of pegvaliase in adolescent subjects with PKU. Subjects in the diet-only control arm will initiate pegvaliase treatment beginning Week 73 and, from Weeks 73 through 145, follow the same dosing and assessment schedule that the active subjects followed from Weeks 1 through 73, after which they will receive pegvaliase for up to 8 additional weeks (through Week 153).

Eligible subjects are required to have 2 blood Phe concentration measurements > 600 µmol/L during the Screening/Run-in Period (7 to 10 days apart) and an average blood Phe concentration > 600 µmol/L over the past 12 months prior to Screening based on available historical data. Once enrolled, patients will be dosed with pegvaliase according to the I/T/M dosing regimen based on the US package insert (USPI), with the guidance to increase dose depending on individual blood Phe lowering efficacy (per investigator's discretion). Dose should be increased to 40 mg/day if blood Phe is > 360 µmol/L after 24 weeks dosing at 20 mg/day, and increased to 60 mg/day if blood Phe is > 360 µmol/L after 16 weeks dosing at 40 mg/day. If a subject's blood Phe is > 600 µmol/L after 16 weeks of continuous treatment with pegvaliase at 60 mg/day, pegvaliase should be permanently discontinued.

BioMarin recommends that the target blood Phe levels should be based on individual blood Phe lowering efficacy.

In Part 1 (through the Week 73 visit), randomized subjects in both the control (diet-only) and the active (pegvaliase) treatment arms will be required to maintain the same level of dietary protein intake they established during Screening/Run-in. In Part 2, subjects in the control (diet-only) arm must continue to maintain this prescribed level of protein intake, whereas subjects randomized to the active (pegvaliase) arm whose blood Phe concentration is ≤ 360 µmol/L for a minimum of 4 weeks may adjust dietary protein intake based on individual subject response to pegvaliase and guidance from the investigator.

4.2 Scientific Rationale for Study Design

This Phase 3 multi-center study is designed to evaluate the safety and efficacy of pegvaliase administered daily to adolescent subjects with PKU. Approximately 54 US and ex-US subjects (ages 12 to 17 years old (US), inclusive, and 12 to 15 years old (ex-US), inclusive) will be evaluated using an open-label, 2-arm, randomized control design, with diet-only management of PKU as the control.

A RCT design, with diet-only management of PKU as the control, will allow for robust data collection and minimize the risk of confounding factors influencing the results. An open-label diet-only control arm is the best comparator for this age group as daily placebo injections would place undue burden on adolescent PKU patients.

Except for subjects randomized to pegvaliase in Part 2 whose blood Phe concentration is ≤ 360 µmol/L for a minimum of 4 weeks, subjects in both the control (diet-only) and active (pegvaliase) treatment arms are required to maintain a consistent level of protein intake throughout the study (see Section 4.1 and Section 8.1.2). Subjects are required to maintain a consistent level of protein intake to ensure that the safety and efficacy endpoints are attributable to pegvaliase rather than to changes in dietary protein intake.

Pegvaliase will be subcutaneously injected in a hospital setting for the first 8 weeks and for all subsequent dose escalations, and afterwards at the clinic or at home by a trained subject, caregiver, adult observer, or visiting healthcare provider. Administration at home makes daily treatment less burdensome for subjects, thereby improving treatment compliance.

An adult observer is required for a minimum of 1 hour post injection throughout the study. An adult observer is defined as someone who will be present with the subject during and after pegvaliase administration, is able to recognize the signs and symptoms of an anaphylaxis episode, and is able to call for emergency medical support and administer epinephrine, if warranted. The requirement for an adult observer was added to ensure that

subjects self-administering pegvaliase would have help in recognizing and quickly responding to signs and symptoms of anaphylaxis should they emerge.

The primary efficacy endpoint is the change from baseline in blood Phe following 72 weeks on study (the end of Part 1). Blood Phe level is the established clinical marker used to diagnose PKU and assess disease control, and reduction of blood Phe concentration to target levels is the universally acknowledged treatment goal for PKU patients. Published literature support the clinical benefit of lowering blood Phe concentration to ≤ 600 µmol/L for associated improvement in clinical outcomes including cognitive performance, behavior and mood (Palermo 2017; Romani 2017; Pietz 1997; Azen 1996; Smith 1989; Fitzgerald 2000; Costello 1994; Fonnesbeck 2013; Gassio 2003).

In addition, evidence has shown improved clinical benefit with even more aggressive blood Phe lowering targets. In children and adolescents with PKU, studies show that patients with blood Phe levels < 360 µmol/L perform better at neuropsychological and neurocognitive tasks (Huijbregts 2002; Jahja 2014). The findings from these studies demonstrate the need to adequately control blood Phe levels in PKU pediatric patients to below the 600 µmol/L threshold.

4.2.1 Subject Input into Design

BioMarin sought input from the PKU community in designing this study. Parents and a scientist from the National PKU Alliance reviewed portions of the protocol and provided feedback. Among other important input, the community stressed the importance of:

- Setting expectations for study participation, something that is critical to parents during the informed consent process
- Ongoing support from site personnel during the study
- Training for adult observers
- Simplifying, as much as possible, the diet tracking requirements given the time commitment required on the part of families
- Flexibility in scheduling clinic visits and including home and virtual visits where possible

BioMarin has taken this PKU community feedback into account when designing the study:

- Detailed training will be provided for subjects, parents, and adult observers, and will continue throughout the study.
- Diet diaries will be in paper format and, consistent with past studies, diet tracking is restricted to the 3 days prior to study visits.

- Scheduled visits will take place at the clinic or, with the approval of the investigator or designee, virtually. For virtual visits the investigator/designee performs assessments while online with the subject, apart from specimen collections and vital signs, which are performed by home healthcare personnel.

4.3 Justification for Dose and Dose Regimen

Pegvaliase is a daily treatment for patients with PKU that will be administered in an Induction/Titration/Maintenance (I/T/M) regimen described in Section 6.5. Previous clinical studies have suggested the primary clearance mechanism of pegvaliase from plasma is via formation of circulating immune complexes leading to complement activation and removal of the drug by phagocytosis, indicating pegvaliase plasma exposure was driven by immune response. Considering that the immune system matures prior to adolescence (Olin 2018; Georgountzou 2017), the immune response to pegvaliase among adolescent subjects is expected to be similar to the responses observed in 16 to 57-year-old study subjects in the pegvaliase clinical development program.

Following dosing in a hospital setting for the initial doses (first 8 weeks) and apart from subsequent dose escalations to 20, 40, and 60 mg/day, which will also be administered in a hospital setting, pegvaliase will typically be administered at home by the trained subject, though pegvaliase may be also administered at home by a trained caregiver or visiting healthcare provider. In addition to training in drug administration prior to the first in-clinic dose, subjects, caregivers, and adult observers will receive extensive training on how to monitor and respond to possible AEs (including acute systemic hypersensitivity reaction [anaphylaxis]) that may be associated with study drug treatment.

Pegvaliase will be administered using an I/T/M dosing regimen similar to the regimen used in Phase 3 studies performed in the US (165-301 and 165-302) and included in the USPI and SmPC (see Table 6.5.1 and Figure 1.2.2). During pegvaliase clinical development, this dosing regimen was found to help mitigate the onset and severity of hypersensitivity reactions while substantially reducing blood Phe concentrations.

The pegvaliase dose levels in this study have been evaluated in 6 multiple-dose Phase 2 and Phase 3 studies. The dose level for induction in 165-306 will be 2.5 mg once weekly and was chosen because this dose with a once weekly dosing frequency administered during the initial 4 weeks of treatment (ie, induction) used in Studies PAL-002, 165-205, and 165-301 has been associated with a lower incidence and severity of hypersensitivity reactions when compared with 5 days per week dosing frequency at a higher starting dose (0.4 mg/kg) studied in Study PAL-004.

4.4 End of Study Definition

A subject is considered to have completed the study if he/she has completed all scheduled visits, including procedures scheduled at the Study Completion/Early Termination (SC/ET) visit, as shown in the SoA (see Section 1.3), or the study is closed by the sponsor. The end of the study is defined as the date of the last visit of the last subject in the study.

5 STUDY POPULATION

Prospective approval of protocol deviations to eligibility criteria, also known as protocol exemptions, is not permitted. Rescreening is allowed for out of range laboratory values.

5.1 Inclusion Criteria

Subjects are eligible to be included in the study only if all of the following criteria apply:

Age

1. Is 12 to 17 years old (US), inclusive, or 12 to 15 years (ex-US), inclusive, at the start of the Screening/Runin Period (Day 28).

Type of Participant and Disease Characteristics

2. Diagnosis of PKU and failure to maintain recommended blood Phe levels on existing management (sapropterin dihydrochloride and Pherestricted diet) demonstrated by 2 blood Phe concentration measurements > 600 µmol/L during the Screening/Runin Period (7 to 10 days in between blood Phe assessments) and average blood Phe concentration > 600 µmol/L over the past 12 months (per available data).
3. Willing and able to maintain and adjust dietary and medical protein food intake according to the study protocol under the supervision of a study dietician or adequately trained designee per investigator discretion during study participation.
4. If on medication for ADHD, depression, or other psychiatric disorder, stable dose of medication for ≥ 8 weeks prior to enrollment and willing to maintain stable dose unless a change is medically indicated.
5. An adult (≥ 18 years of age) has been identified who is willing and competent to observe the subject during study drug administration and for a minimum of 1 hour following administration.
6. Able and willing to comply with all study procedures.
7. Generally in good health, as evidenced by physical examination, clinical laboratory evaluations (hematology, chemistry, and urinalysis), and electrocardiogram (ECG) tests performed at Screening.

Sex and Contraceptive/Barrier Requirement

8. Males and females are eligible to participate in this clinical study.
9. Females with childbearing potential must have a negative pregnancy test at Screening/Run-in and be willing to have additional pregnancy tests during the study.
10. If sexually active, male or female subjects must not plan to become pregnant (self or partner) and must use 2 acceptable methods of contraception while participating in the study beginning at Screening and for 4 weeks after discontinuing study drug.

Contraceptive use must be consistent with the guidance provided in Appendix 4, Section 10.4 of this protocol. Acceptable methods of contraception may include:

- Primary forms: hormonal (combination hormone-containing pills, patch, vaginal ring, or intrauterine device) or non-hormonal (copper-containing intrauterine device).
- Secondary forms: barrier forms and other forms of birth control and must include spermicide (eg, male condom; female condom is not an acceptable secondary form).

11. Contraceptive use should be consistent with local regulations regarding the methods of contraception for those participating in clinical studies.

Informed Consent

12. Subjects must be capable of giving signed informed consent as described in Appendix 1, Section 10.1, which includes compliance with the requirements and restrictions listed in the informed consent form (ICF) and in this protocol. In the case of subjects under the age defined by regional law or regulation, parent(s) or guardian(s) must be willing and able to provide written, signed informed consent after the nature of the study has been explained and prior to performance of any researchrelated procedure. Subjects under the age of majority must be willing and able to provide written assent (if required by local regulations or the IRB/IEC) after the nature of the study has been explained and prior to performance of any -researchrelated- procedure.

5.2 Exclusion Criteria

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

Medical Conditions:

1. Have a history of a severe systemic hypersensitivity reaction or recurrence of a mild to moderate acute systemic hypersensitivity reaction to any of the excipients or another PEGylated medicinal product.
2. Concurrent disease or condition that would interfere with study participation or safety (eg, history or presence of clinically significant cardiovascular, pulmonary, hepatic, renal, hematologic, gastrointestinal, endocrine, immunologic, dermatologic, neurological, oncologic, or psychiatric disease).

Prior/Concomitant Therapies:

3. Taking any of the prohibited medications outlined in Section 6.8.1.
4. Previous treatment with pegvaliase.
5. Use of any medication that is intended to treat PKU, including the use of large neutral amino acids, within 14 days prior to the administration of study drug on Day 1.

6. Use or planned use of any injectable drugs containing polyethylene glycol (PEG; other than pegvaliase), including medroxyprogesterone injection, within 3 months prior to the start of Screening/Runin and during study participation with the exception of- COVID-19 vaccinations (Section 6.8.1).
7. A history of organ transplantation or on chronic immunosuppressive therapy.

Prior/Concurrent Clinical Study Experience

8. Use of any investigational product or investigational medical device within 30 days prior to Screening/Run-in or requirement for any investigational agent prior to completion of all scheduled study assessments.

Diagnostic Assessments

9. A positive test for HIV antibody, hepatitis B surface antigen, or hepatitis C antibody.
10. Alanine aminotransferase (ALT) concentration > 2 × the upper limit of normal (ULN).
11. Creatinine > 1.5 × ULN.

Other Exclusions

12. A history of substance abuse (as defined by the American Psychiatric Association: Diagnostic and Statistical Manual of Mental Disorders [DSM]) in the past 12 months or current alcohol or drug abuse.
13. Pregnant or breastfeeding or planning to become pregnant (self or partner) or breastfeeding at any time during the study.
14. Major surgery planned during the study period.
15. Inability to identify and/or communicate to others that the subject is experiencing symptoms of potential anaphylaxis due to cognitive impairment or other reasons.
16. Unwillingness or inability of subject/caregiver to carry auto-injectable epinephrine with them at all times.
17. Any condition that, in the view of the investigator, places the subject at high risk of poor treatment compliance or terminating early from the study.

5.3 Lifestyle Considerations

5.3.1 Meals and Dietary Restrictions

Details of all PKU-specific dietary therapy (eg, Phe intake, amino-acid supplementation) must be recorded in the source document and electronic case report form (eCRF).

Dietary Instruction

Subjects must maintain dietary Phe intake levels that are consistent with their baseline levels for the duration of the study unless clinically indicated, per dietician or adequately trained

designee per investigator/designee discretion to support individual nutritional needs during study participation (refer to Section 8.1.2).

Fasting Requirements

Fasting (water only) is required for at least 2.5 hours prior to any blood Phe measurement.

Any time fasting is required for visit assessments, the fasting assessments should be completed and fasting stopped before clinical outcome assessments are performed. The fast can be broken with a snack or meal as preferred.

5.3.2 Activity Restrictions

Subjects should abstain from strenuous exercise for 48 hours before each blood collection for clinical laboratory tests. Subjects may participate in light recreational activities (such as watching television or reading) prior to study visits, as appropriate.

5.4 Screen Failures

Screen failures are defined as subjects who assent to participate (and whose parents/guardians consent to their participation) in the clinical study but who are not subsequently enrolled. A minimal set of screen failure information is required to ensure transparent reporting of screen failure subjects to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and the occurrence of any serious adverse event (SAE).

Individuals who do not meet the criteria for participation in the study may be rescreened. Rescreened subjects should be assigned the same subject number as for the initial screening. If eligibility criteria are met on retest within the screening window, the subject may be enrolled after confirmation by the medical monitor. Subjects who do not meet eligibility criteria within the screening window, will have to perform all Screening Visit procedures and assessments when re-screened.

5.5 Study Stopping Criteria

The independent Data Monitoring Committee (DMC) will be responsible for performing a benefit to risk assessment of pegvaliase in this patient population on a regular (quarterly) and ad hoc basis during the study and for making recommendations to BioMarin with regards to study conduct. The DMC Chair will be informed of all subjects meeting the Individual Stopping Criteria Evaluation for Adverse Events During Pegvaliase Treatment (Section 8.3.8.1.1).

6 STUDY TREATMENT

6.1 Study Treatment(s) Administered

Study treatment is defined as any investigational treatment(s), marketed product(s), placebo, or medical device(s) intended to be administered to a study subject according to the study protocol.

The investigational treatment is pegvaliase (recombinant *Anabaena variabilis* phenylalanine ammonia lyase-PEG). For subcutaneous administration, subjects are supplied with prefilled syringes in 3 sizes. Sizes are 2.5 mg (0.5 mL of 5 mg/mL protein concentration), 10 mg (0.5 mL of 20 mg/mL protein concentration), and 20 mg (1.0 mL of 20 mg/mL protein concentration).

The excipients in this drug product are tromethamine, tromethamine-hydrochloride, sodium chloride, trans-cinnamic acid, and water for injection.

Study treatment details are provided in Table 6.1.1.

Table 6.1.1: Study Treatment

Treatment Name	pegvaliase
Type	drug
Dose Formulation	prefilled syringe
Unit Dose Strength	Each PFS is filled to deliver at least 0.5 or 1 mL of formulation containing 2.5, 10, or 20 mg of pegvaliase
Dosage Level(s)	2.5 mg (1x or 2x per week), 10 mg (2x or 4x per week), 20 mg (daily), 40 mg (daily), 60 mg (daily)
Route of Administration	subcutaneous injection
IMP and NIMP	IMP
Sourcing	provided centrally by the sponsor
Packaging and Labeling	Drug product packaging is identified with lot and identification numbers and provided in a box labeled with the study number and identified to be for investigational use.
Current/Former Name(s) or Alias(es)	pegvaliase, BMN 165, rAvPAL-PEG (PEGylated recombinant Anabaena variabilis phenylalanine ammonia lyase), Palynziq® (trade name)

IMP, investigational medical products; NIMP, non-investigational medical products; PFS, pre-filled syringe.

In addition to pegvaliase, treatment in this study includes a diet in which total protein intake (defined as the sum of protein intake from medical food and intact food) must remain stable and consistent with baseline levels. Specifically, to accurately assess the primary endpoint comparison between the active (pegvaliase) and control (diet-only) treatment arms on the

change from baseline in blood Phe concentration following 72 weeks on study, total protein intake must remain stable and consistent with baseline levels for subjects in both treatment arms through Part 1. In Part 2, subjects in the control arm must continue to maintain this prescribed level of protein intake, whereas subjects randomized to pegvaliase whose blood Phe concentration is ≤ 360 µmol/L for a minimum of 4 weeks may adjust dietary protein intake based on individual subject response to pegvaliase and guidance from the investigator. Details on diet monitoring is provided in Section 8.1.2.

6.1.1 Additional Drug Administration Instructions

Each refilled syringe is intended for a single subcutaneous injection. The recommended injections sites include the front middle of thighs and the abdomen at least 2 inches (5 centimeters) away from the navel. If a caregiver is giving the injection, the top of the buttocks and the back of the upper arms are also appropriate injections sites. The injection sites for administration of pegvaliase should alternate between doses. Pegvaliase must not be injected into moles, scars, birthmarks, bruises, rashes, or areas where the skin is hard, tender, red, damaged, burned, inflamed, or tattooed. If multiple injections are needed for a single dose, injections should be administered at the same time of day and injection sites should be at least 5 cm away from each other. Doses should not be divided over the course of the day.

A subject (or subject-designated caregiver) must meet all of the following criteria to be eligible to administer the drug:

- Has no known cognitive impairments that may increase the safety risk per investigator assessment
- Has no medical history or current medications that may compromise administration of pegvaliase per investigator assessment
- Has completed all required drug administration training and has demonstrated administration competency per investigator assessment, including how to prepare pegvaliase for administration and safely perform the injection

Refer to Section 6.5 for study drug administration requirements regarding the presence of an adult observer and for premedication requirements.

Following a dose interruption of ≥ 4 doses, the investigator should obtain approval from the medical monitor prior to the subject restarting pegvaliase. Dose regimens may be revised provided the investigator obtains approval from the medical monitor prior to starting an alternative dose regimen.

The suitability for self-administration of pegvaliase will be reassessed by the investigator if a subject experiences any issues with self-administration. In addition, if a subject has an

NCI-CTCAE grade 3 or higher AE during the study, the subject's suitability for self-administration of pegvaliase will be reassessed by the investigator.

Between scheduled clinic visits, the clinic staff will contact the subject weekly to monitor if the subject is experiencing problems with drug administration, for any AEs, and to answer questions throughout the duration of the study. The subject must perform the scheduled assessments and procedures in the clinic as outlined in the SoA (Section 1.3). Training materials and other information specific to the study site personnel will be provided in the study reference materials, which includes drug administration training materials and instruction on the following:

- How to prepare and safely perform the injections of pegvaliase
- How to receive, store, and return both used and unused pegvaliase
- How to safely use and dispose of prefilled syringes used for injections of pegvaliase
- How to use a prefilled syringe every time drug is administered
- How to care for the injection site after an injection of pegvaliase
- How to document each pegvaliase injection
- How to identify an AE and how to report this to the study site
- How and when to use epinephrine
- Whom to contact at the study site in case of an emergency

Subjects will be provided with a workbook to document the date and time of pegvaliase injections, injection site, any suspected AEs, and other relevant information. Dosing in response to an AE should be determined by the investigator.

6.2 Preparation/Handling/Storage/Accountability

At the study site, all study drug must be stored at 5 ±3°C (41 ±5°F), under the conditions specified in the IB, and in a secure area accessible only to the designated pharmacists and clinical site personnel. All study drug must be stored and inventoried, and the inventories must be carefully and accurately documented according to applicable state, federal, and local regulations; ICH Good Clinical Practice (GCP); and study procedures. Information regarding storage of study drug in subjects' homes is provided in the study training materials.

The investigator or designee must confirm appropriate temperature conditions have been maintained during transit for all study treatment received and that any discrepancies are reported and resolved before use of the study treatment.

Only subjects enrolled in the study may receive study treatment, and only authorized site staff may supply study treatment. All study treatment must be stored in a secure, environmentally controlled and monitored (manual or automated) area in accordance with the labeled storage conditions, with access limited to the investigator and authorized site staff.

The investigator, institution, qualified pharmacist, or the head of the medical institution (where applicable) is responsible for study treatment accountability, reconciliation, and record maintenance (ie, receipt, reconciliation, and final disposition records).

Subjects are provided instructions for returning used and unused study drug prefilled syringes and for proper disposal at each site visit.

Further guidance and information for the final disposition of unused study treatments are provided in the Pharmacy Manual.

6.3 Measures to Minimize Bias: Randomization and Blinding

This is an open-label study; however, the specific intervention to be taken by a subject will be assigned using an IVRS/IWRS. The site will contact the IVRS/IWRS prior to the start of study intervention administration for each subject. The site will record the intervention assignment on the applicable case report form, if required. Potential bias will be reduced by central randomization.

6.4 Study Treatment Compliance

When subjects are dosed at the site, they will receive study treatment directly from the investigator or designee, under medical supervision. The date and time of each dose administered in the clinic will be recorded in the source documents and recorded in the eCRF. The dose of study treatment and study subject identification will be confirmed at the time of dosing by a member of the site staff other than the person administering the study treatment.

When subjects or caregivers administer study treatment(s) at home, the date, time, and quantity of each dose of study drug administered to each subject must be recorded by the subject or caregiver in a workbook provided for the study. The site staff will collect the workbook at scheduled clinic visits and transcribe this information to the appropriate eCRF.

These data will be used to assess compliance. Deviation(s) from the prescribed dosage regimen should be recorded in the eCRF. Treatment start and stop dates, including dates for treatment delays and/or dose reductions, should also be recorded in the eCRF.

6.5 Dose Modification

Subjects will receive pegvaliase at a concentration of 5.0 or 20.0 mg/mL. Pegvaliase will be administered subcutaneously at dose levels in the range of 2.5 to 60 mg. The minimum dose of pegvaliase is 2.5 mg/week. The maximum allowable daily dose of pegvaliase is 60 mg/day (for a maximum weekly total dose of 420 mg).

The duration of the Primary Treatment Phase (Part 1) is 72 weeks, during which pegvaliase treatment will be initiated for subjects randomized to the active (pegvaliase) treatment arm using an I/T/M dosing regimen. The recommended dosing schedule for subjects dosing with pegvaliase is shown in Table 6.5.1. Induction will consist of a 4-week period when subjects randomized to the active treatment arm will receive pegvaliase subcutaneously with a PFS at a fixed dose of 2.5 mg/week and during which the dosing regimen will not be modified in response to blood Phe concentration. Once subjects complete induction they will titrate up to a dose of 10 mg/day. The dosing frequency will be increased gradually during titration dosing to daily (7 days/week). During Maintenance Dosing the dose will be increased to 20 mg/day, 40 mg/day, and 60 mg/day depending upon individual blood Phe lowering efficacy (per the investigator's discretion). The dose should be increased to 40 mg/day if blood Phe is > 360 µmol/L after 24 weeks dosing at 20 mg/day and increased to 60 mg/day if blood Phe is > 360 µmol/L after 16 weeks dosing at 40 mg/day. If a subject's blood Phe is > 600 µmol/L after 16 weeks of continuous treatment with pegvaliase at 60 mg/day, pegvaliase should be permanently discontinued. BioMarin recommends that the target blood Phe levels should be based on individual needs, with a target blood Phe ≤ 600 µmol/L.

Duration between dose titration steps may be increased in response to AEs. Dose level reductions due to AEs or hypophenylalaninemia (confirmed with 2 consecutive blood Phe values < 30 µmol/L) may be made at any time during the study at the discretion of the investigator. Phe levels will be regularly monitored and adjustments in the pegvaliase dose and/or dietary intake of Phe will be required to mitigate the potential risk of chronic hypophenylalaninemia levels (< 30 µmol/L) in adolescent subjects.

Following completion of Part 1, subjects randomized to the active (pegvaliase) treatment arm will enter the Extension Phase (Part 2), during which subjects will continue dosing with pegvaliase at their optimal maintenance dose level for up to an additional 80 weeks in order to evaluate the long-term safety and efficacy of pegvaliase in adolescent subjects with PKU.

Following completion of Part 1, subjects randomized to the control (diet-only) treatment arm in Part 1 will repeat the Part 1 assessment schedule while initiating pegvaliase dosing from Weeks 73 through 145, after which they will receive pegvaliase for up to 8 additional weeks (through Week 153).

Table 6.5.1: Study Drug Dose Titration Schedule

Part 1 Dosing	Duration [a]	Dose (mg)	Frequency of Administration (Doses Per Week)
Induction	4 weeks	2.5	1
Titration	5 weeks	2.5	2
		10	1
		10	2
		10	4
		10	7
Maintenance	20 mg daily: 24 weeks [b] 40 mg daily: 16 weeks [b,c] 60 mg daily: maximum dose [c]	20	7
		40	7
		60	7

[a] Minimum duration prior to dose escalation. Dose escalation may be delayed depending on subject tolerability.

[b] Prior to initial dose escalation, the minimum amount of time at 20 mg/day is 24 weeks and the minimum amount of time at 40 mg/day is 16 weeks. The dose should be increased to 40 mg/day if blood Phe is > 360 µmol/L after 24 weeks dosing at 20 mg/day, and increased to 60 mg/day if blood Phe is > 360 µmol/L after 16 weeks dosing at 40 mg/day.

[c] If multiple injections are needed for a single dose, the injections should be administered at the same time of day and injection sites should be at least 5 cm away from each other. Doses should not be divided over the course of the day. If a subject's blood Phe is > 600 µmol/L after 16 weeks of continuous treatment with pegvaliase at 60 mg/day, pegvaliase should be permanently discontinued.

Following enrollment, subjects (and an adult caregiver) will be trained on how to administer study drug at home. Pegvaliase dosing will be conducted in a hospital setting for at least the first 8 weeks including titration up to 10 mg once daily, and for all subsequent dose escalations. The subject (and the adult caregiver) must demonstrate competency with injecting study drug in order to be permitted to administer study drug at home. Dosing at home will not be allowed until the principal investigator has assessed the subject's and adult observer's competency in proper study drug administration and recognition and appropriate management of the potential risks.

Throughout the study, an adult observer must be present during study drug administration and for a minimum of 1 hour following administration. An adult observer is defined as someone who will be present with the subject during and after pegvaliase administration, is able to recognize the signs and symptoms of an anaphylaxis episode, and is able to call for emergency medical support and administer epinephrine, if warranted. Administration of study drug may only be performed if this adult observer is present. Subjects and the adult observers who are observing them during study drug administration will be provided with information and extensive training regarding how to recognize a possible allergic reaction,

the severity of the reaction, and instructions on what to do if a reaction occurs. Home administration may not performed until the investigator has assessed the adult observer's competency in proper study drug administration and the recognition and appropriate management of potential risks.

Subjects will be provided with 2 epinephrine injectors and are required to carry both injectors with them at all times. Subjects and adult observers will be trained to recognize the signs and symptoms of anaphylaxis and, if these reactions occur, to call for emergency medical support and administer an epinephrine injector. For the duration of the study, each subject will be contacted weekly by study site personnel to monitor for administration problems and AEs (see Section 8.3.8.1 for details on the management of suspected anaphylaxis in this study).

All subjects receiving pegvaliase must be premedicated with an H1 antagonist, and an H2 antagonist, and an antipyretic approximately 2 to 3 hours prior to each dose until they reach the Maintenance Phase, defined as when subjects achieve blood Phe ≤600 µmol/L while they are on a stable dose of pegvaliase. Premedication may be considered during the Maintenance Phase at the investigator's discretion, eg, approximately 2 to 3 hours prior to study drug for 1 week upon reintroduction of study drug, upon resolution of an AE, following any dose interruption of ≥ 4 days, and for any dose increases in the Extension Phase. Subjects may also be premedicated at any other time in the study per investigator discretion.

This premedication regimen was successfully implemented in the Phase 3 adult clinical program. Subjects will be provided with a workbook to document the date and time of study drug injections, the injection site, premedication use, and suspected AEs.

If ≥ 4 consecutive doses are missed for reasons other than safety related issues, the investigator should obtain approval from the medical monitor prior to the subject resuming study drug.

6.5.1 Dosing Modifications in Response to Hypersensitivity Adverse Events

Dosing modifications in response to a hypersensitivity adverse event (HAE) depends on the NCI-CTCAE grade and suspected relationship study drug. Dosing instructions are provided in Table 6.5.1.1 (regardless of previous occurrence). Dosing modifications are per reported HAE, independent of prior reported HAEs.

Table 6.5.1.1: Dosing Instructions in Response to a Hypersensitivity Adverse Event

NCI-CTCAE Grade [a]	Related to Study Drug	Action with Study Drug			Individual Stopping Criteria Evaluation [d]	HRV Assessment [e]
		Maintain [b]	Reduce [c]	Interrupt [c]		
1	Yes or No	X	(X) Optional	(X) Optional		Investigator discretion
2	Yes or No	X	(X) Optional	(X) Optional		Investigator discretion
3	No				X Immediately consult with sponsor medical monitor	Investigator discretion
3 [d]	Yes			X	X Immediately consult with sponsor medical monitor	Yes (if within 24 hours of onset)
4 [d]	Yes or No			X	X Immediately consult with sponsor medical monitor	Yes (if within 24 hours of onset)

AE, adverse event; CTCAE, Common Terminology Criteria for Adverse Events, Version 5.0; HRV, Hypersensitivity Reaction Visit; NCI, National Cancer Institute.

[a] NCI-CTCAE grade determination is performed by the investigator and may be done either via telephone or clinic visit.

[b] The investigator will instruct the subject to maintain the study drug dose at the time of AE onset until improvement to Grade 1 or resolution (per investigator assessment in the clinic or via telephone).

[c] The study drug dose may be reduced or interrupted if necessary per investigator determination.

[d] If a subject has an NCI-CTCAE Grade ≥ 3 hypersensitivity AE that is related to study drug and is suspected to meet Brown's criteria for severe (Grade 3) in the judgment of the investigator and/or the sponsor's medical monitor, the subject will be permanently discontinued from study drug.

[e] If the investigator determines that the NCI-CTCAE Grade ≥ 3 hypersensitivity reaction is related to administration with study drug, the subject will be asked to return to the clinic within 24 hours of event onset for evaluation, including laboratory tests (chemistry, hematology, urinalysis, anti-pegvaliase IgE [sampling should be performed 8 to 24 hours after event onset and before the next dose of study drug], urine albumin/creatinine ratio, CRP, C3, and C4).

Once an HAE (other than an anaphylaxis episode) improves to Grade 1 or resolves, the study drug dose may be increased, maintained, or reduced, at the discretion of the investigator. If reduced, reductions should be from 60 to 40 mg/day, from 40 to 20 mg/day, or from 20 to 10 mg/day, whichever is appropriate.

6.6 Treatment after the End of the Study

There is no treatment following completion of the study.

6.7 Overdose

For this study, any dose of pegvaliase greater than the prescribed dose will be considered an overdose.

BioMarin does not recommend specific treatment for an overdose. In the event of an overdose, the investigator should contact the BioMarin medical monitor immediately.

6.8 Concomitant Therapy

Any medication or vaccine (including over-the-counter or prescription medicines, vitamins, and/or herbal supplements) that the subject is receiving at the time of enrollment or receives during the study must be recorded on the designated eCRF along with:

- Reason for use
- Dates of administration including start and end dates
- Dosage information including dose and frequency

The medical monitor should be contacted if there are any questions regarding concomitant or prior therapy. The investigator may prescribe additional medications, deemed necessary to provide adequate prophylactic or supportive care, during the study, as long as the prescribed medication is not prohibited by the protocol (refer to Section 5.2). In the event of an emergency, any needed medications may be prescribed without prior approval, but the medical monitor must be notified of the use of any contraindicated medications immediately thereafter. Any concomitant medications added or discontinued during the study should be recorded on the eCRF. Medications should, whenever possible, not be recorded in the electronic database with a frequency of PRN.

6.8.1 Excluded Concomitant Medications

The following medications are prohibited starting at the time specified and until the end of the study:

- Any medication that is intended to treat PKU, including the use of large neutral amino acids, within 14 days prior to the administration of study drug on Day 1.
- Any injectable drugs containing polyethylene glycol (PEG; other than pegvaliase), including medroxyprogesterone injection, within 3 months prior to the start of Screening/Runin and during study participation with the exception of COVID-19 vaccinations. Please contact the medical monitor prior to administering COVID-19 vaccinations.

- Use of any investigational product or investigational medical device within 30 days prior to Screening/Run-in or requirement for any investigational agent prior to completion of all scheduled study assessments.

In addition, if a subject is on medication for ADHD, depression, or other psychiatric disorder, the dose must be stable for ≥ 8 weeks prior to enrollment and must continue to be stable throughout the study unless a change is medically indicated.

6.8.2 Permitted and/or Required Concomitant Medications

All prescription and over-the-counter medication including herbal supplements and medical food taken by a subject for 30 days before screening are recorded on the designated CRF. Psychiatric medications and their dosage should remain consistent throughout the study unless a change is medically indicated. No new psychiatric medication should be initiated during the study unless medically indicated. No psychotherapy should be initiated during the study unless medically indicated. The investigator may prescribe additional medications during the study as long as the prescribed medication is not prohibited by the protocol (see Section 6.8.1). In the event of an emergency, any needed medications may be prescribed without prior approval but the medical monitor must be notified of the use of any contraindicated medication immediately thereafter. Any concomitant medication added or discontinued during the study should be recorded on the appropriate CRF.

All subjects receiving pegvaliase will be required to premedicate with an H1 antagonist, and an H2 antagonist, and an antipyretic approximately 2 to 3 hours prior to each dose of study drug until they reach the Maintenance Phase, defined as when subjects achieve blood Phe ≤600 µmol/L while they are on a stable dose of pegvaliase. Premedication may be considered during the Maintenance Phase at the investigator's discretion. For further information regarding premedication, refer to Section 6.5.

7 DISCONTINUATION OF STUDY TREATMENT AND SUBJECT WITHDRAWAL FROM STUDY

7.1 Discontinuation of Study Treatment

In rare instances it may be necessary for a patient to permanently discontinue study treatment. If a subject's blood Phe is > 600 µmol/L after 16 weeks of continuous treatment with pegvaliase at 60 mg/day, pegvaliase should be permanently discontinued.

If study treatment is permanently discontinued, the subject will be asked to continue to provide consent at the time of treatment discontinuation to remain in the study and undergo the scheduled study assessments. Refer to the SoA for data to be collected at the time of discontinuation of study treatment and follow-up, and for any further evaluations that need to be completed.

If the subject declines to provide consent to remain in the study, the study completion visit assessments should be performed as detailed in the SoA (Section 1.3).

7.2 Subject Withdrawal from the Study

A subject may withdraw from the study at any time at his or her own request, or may be withdrawn at any time at the discretion of the investigator for safety, behavioral, compliance, or administrative reasons.

At the time of discontinuing from the study, if possible, an early termination (ET) visit should be conducted, as shown in the SoA (Section 1.3).

The subject will be permanently discontinued both from the study treatment and from the study at that time.

If the subject withdraws consent for disclosure of future information, the sponsor may retain and continue to use any data collected before such a withdrawal of consent.

If a subject withdraws from the study, BioMarin will only use the remaining samples to complete study procedures as described in Section 8 and no new samples will be collected. If a subject no longer wants the remaining samples to be used for future research, he/she shall inform the study investigator or institution accordingly and BioMarin will only use the remaining samples for health authority requests related to the activities in Section 8, not for future research. The investigator must document this in the site study records.

7.3 Lost to Follow-Up

A subject will be considered lost to follow-up if he or she repeatedly fails to return for scheduled visits and cannot be contacted by the site.

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

- The site must attempt to contact the subject to reschedule the missed visit as soon as possible, counsel the subject on the importance of maintaining the assigned visit schedule, and ascertain whether or not the subject wishes to and/or should continue in the study.
- Before a subject is deemed lost to follow up, the investigator or designee must make every effort to regain contact with the subject (where possible, 3 telephone calls and, if necessary, a certified letter to the subject's last known mailing address or local equivalent methods). These contact attempts should be documented in the subject's medical record.
- Should the subject continue to be unreachable, he or she will be considered to have withdrawn from the study.

Discontinuation of specific sites or of the study as a whole are handled as part of Appendix 1, Section 10.1.9.

8 STUDY ASSESSMENTS AND PROCEDURES

An ICF must be signed and dated by the subject's legally authorized representative, the investigator or designee, and a witness (if required) before any study-related procedures are performed.

All screening evaluations must be completed and reviewed to confirm that potential subjects meet all eligibility criteria. The investigator will maintain a screening log to record details of all subjects screened and to confirm eligibility or record reasons for screening failure, as applicable. Procedures conducted as part of the subject's routine clinical management (eg, blood count) and obtained before signing of the ICF may be utilized for screening or baseline purposes provided the procedures met the protocol-specified criteria and were performed within the time frame defined in the SoA.

Potential subjects will enter the Screening/Run-in period, which will last 4 weeks and include 2 assessments of blood Phe concentration 7 to 10 days apart. Diet diaries should be completed for the 3 days preceding each of the blood Phe assessments. Following confirmation that the 2 blood Phe results are > 600 µmol/L and all other eligibility criteria have been met, subjects may be enrolled in the study on Day 1. Protocol exemptions for eligibility are not allowed. Following enrollment, subjects will be randomized 2:1 to the active (pegvaliase) or control (diet-only) treatment arm of the study.

In the Primary Treatment Phase (Part 1), subjects in the active (pegvaliase) and control (diet-only) treatment arms will follow the same visit schedules and perform the same assessments except that the control subjects will not be receiving pegvaliase so will not have PK draws or immunogenicity assessments. The timing of Part 1 assessments for active and control subjects is provided in Table 1.3.1 and Table 1.3.3, respectively.

The primary efficacy endpoint, the change from baseline blood Phe, is assessed following 72 weeks on study (the end of Part 1). Beginning Week 73, subjects in the active treatment arm will continue to receive pegvaliase in the Extension Phase (Part 2) for up to an additional 80 weeks (through Week 153). The timing of Part 2 assessments for active treatment subjects is provided in Table 1.3.2.

Subjects in the control (diet-only) treatment arm will initiate pegvaliase treatment beginning Week 73 and, from Weeks 73 through 145, will follow the same dosing and assessment schedule that the active subjects followed from Weeks 1 through 73, after which they will receive pegvaliase for 8 additional weeks (through Week 153). The timing of Part 2 assessments for control subjects is provided in Table 1.3.4. All the assessments, dietary rules, and other restrictions that applied to active subjects in the Primary Treatment Phase (Part 1)

apply to control subjects in Part 2 until the Week 145 visit when change from baseline blood Phe for these subjects will be assessed after 73 weeks of pegvaliase treatment.

The last scheduled visit, which includes study drug administration, is at Week 153 for subjects randomized to both the active (pegvaliase) and control (diet-only) treatment arms. Subjects should perform the Study Completion (SC) Visit 4 weeks after the last dose of study drug or after completion of study participation, whichever occurs last. For subjects who terminate from the study early, the Early Termination (ET) visit should occur within 4 weeks after the last study drug dose or after withdrawal from study participation, whichever occurs last. If study drug is discontinued before study completion, the investigator will ask the subject to remain in the study to continue study visits and assessments, provided the subject's health, safety, and welfare would not be detrimentally affected.

Immediate safety concerns should be discussed with the sponsor (medical monitor) immediately upon occurrence or awareness to determine if the subject should continue or discontinue study treatment.

Adherence to the study design requirements, including those specified in the SoA, is essential and required for study conduct.

8.1 Efficacy Assessments

Planned timepoints for all efficacy assessments are provided in the SoA (refer to Section 1.3).

8.1.1 Blood Phe Level

The primary efficacy endpoint is change in blood Phe concentration from treatment naïve baseline following 72 weeks on study (see Section 9.4.2). Blood samples for blood Phe analysis are to be drawn according to the SoA (see Section 1.3). Monitoring of blood Phe levels is the cornerstone of diagnosis and treatment of PKU patients. Guidelines for the treatment of PKU are based on blood Phe levels (Vockley 2014; van Wegberg 2017), and clinicians and patients use blood Phe concentration to assess and monitor PKU disease progression and control.

Subjects must fast for at least 2.5 hours prior to blood Phe assessments to lessen the effect of food intake on blood Phe concentration. Samples for blood Phe concentration will be analyzed by a central laboratory.

8.1.2 Total Dietary Protein Intake, Dietary Monitoring, and Dietary Protocol Restrictions

Protein from medical foods (Phe-free amino acid fortified food sources) and protein from intact foods (any other food sources containing Phe) are collectively referred to as total dietary protein. Although total dietary protein intake is not an efficacy endpoint in this study, it is an important outcome measure that must remain stable for the primary endpoint to be accurately assessed and under the supervision of a dietician or adequately trained designee per the investigator during study participation. Specifically, to accurately assess the primary endpoint comparison between the active (pegvaliase) and control (diet-only) treatment arms on the change from baseline in blood Phe concentration following 72 weeks on study, total protein intake must remain stable for subjects in both treatment arms during this period. It is essential that subjects maintain stable protein intake to ensure that the measured changes in their blood Phe are attributable to study treatment rather than changes in their dietary protein intake.

A subject's diet is monitored using a 3-day diet diary. The 3-day dietary record (diet diary) is completed by subjects, brought to clinic visits for review, and maintained with the study source documents. Subjects record all food, beverages, special low-protein foods, and medical foods consumed for 3 consecutive days prior to the scheduled clinic visit and data are analyzed by the nutritional software (Metabolic Pro®) for total kcals, protein, Phe, tyrosine, and the percentage of daily recommended intake provided for protein, Phe, tyrosine, vitamins, and minerals.

A dietitian under investigator supervision is required to monitor and manage the subject's diet for the entire duration of the study, though an investigator or designee may also perform this role. Subjects will be provided with 3-day diaries in which all dietary protein intake (including medical food and/or intact food) must be recorded for 3 consecutive days immediately prior to each scheduled clinic visit for review with the dietitian. The subject may be asked to repeat the 3-day diary collection if the dietitian or investigator/designee considers the diary has not been completed accurately; in such circumstances, study visits will be rescheduled. All subjects will be provided the option of tyrosine supplementation (500 mg, 3 times per day with meals) at the discretion of the investigator.

The change from baseline in total protein intake at Week 73 will be compared as a secondary endpoint. For this endpoint, baseline protein intake is defined as the average of protein intake values derived from diet diaries collected during Screening/Run-in. During Screening/Run-in, 3-day diet diaries are completed 3 times, each time covering the 3 days periods prior to blood Phe assessments (and collected on the day of those assessments). The

3 Screening/Run-in blood Phe assessments are scheduled to occur (1) at Day -25 to Day -23; (2) 7 to 10 days later (ie, in the Day -18 to Day -13 window); and (3) at Day 1 pre-dose. Thus, the 3 day diet assessments will assess diet for (1) the 3 days within the Day -28 to Day -23 window immediately prior to the first blood Phe assessment; (2) the 3 days within the Day -21 to Day -15 window immediately prior to the second blood Phe assessment; and (3) for Days -3 to Day 1.

Subjects will be instructed not to change their total dietary protein intake (medical food and/or intact food) during the Screening/Run-in and Primary Treatment Phase (Part 1) of the study. However, modifications to a subject's diet and reductions in study drug dose level must be implemented if blood Phe levels are reduced to < 30 µmol/L and confirmed upon recheck (performed within approximately 2 weeks) (see Section 8.1.2.1).

In Part 2, dietary protein intake may be modified for subjects randomized to active (pegvaliase) treatment if blood Phe concentration is ≤ 360 µmol/L for a minimum of 4 weeks. Should that occur, subjects may adjust dietary protein intake based on individual subject response to pegvaliase and guidance from the investigator or designee (see Section 8.1.2.2). Subjects randomized to the control (diet-only) treatment arm must continue to maintain dietary protein intake consistent with baseline through Week 145 except in the case of blood Phe levels < 30 µmol/L confirmed upon recheck (see Section 8.1.2.1).

Baseline total dietary protein intake will be used as the comparator for subsequent 3-day diet diary entries. Subjects will be required to maintain dietary protein intake levels that are consistent with their baseline levels for the entire duration of the study, except as otherwise specified above, with a consistent diet defined as one in which the intact protein changes are < 15% from baseline and the medical food protein changes are < 15% from baseline.

If a subject demonstrates ≥ 15% change from baseline intake of total intact protein and/or medical food protein, the dietitian (or investigator/designee) will counsel the subject to resume their baseline diet and may perform another 3-day diet record and blood Phe concentration assessment in 2 weeks. If the subject demonstrates ≥ 15% increase in intake of protein from intact foods and/or medical food at the next scheduled study visit, a discussion with the medical monitor may be required to discuss further actions related to non-adherence with consistent dietary protein intake throughout the study, at the discretion of the investigator.

If dietary protein intake is determined to be inconsistent with baseline values, the study site dietitian (or investigator/designee) should counsel the subject on how to return to baseline values by modifying intake of protein from intact foods and/or medical food. If diet changes have been implemented, a blood Phe concentration assessment and 3-day diet record should

be collected 2 weeks after implementation, in addition to all regularly scheduled assessments. Any changes must also be documented on the appropriate eCRF.

8.1.2.1 Diet Modification for Blood Phe Levels Less than 30 µmol/L

In addition to protocol required dose reductions (Section 6.5), modifications to subject's diet should be performed if blood Phe levels are < 30 µmol/L and confirmed upon repeat blood Phe measurement (performed within approximately 2 weeks). Subjects will receive counseling for modification of intake of intact protein and medical foods based on intake at the time of blood Phe assessment, as follows:

- If it is determined that a subject is consuming < 2x their recommended level of intact protein per Dietary Reference Intake (DRI) guidelines (1.6 g/kg/day), the dietitian may instruct the subject to increase their intact protein by 20 grams/day and decrease their medical food protein by 20 grams/day.
- If it is determined that a subject is consuming ≥ 2x their recommended level of intact protein per DRI guidelines, the dietitian may instruct the subject to increase their intact protein by 10 grams/day and decrease their medical food protein by 10 grams/day.

Further increases in protein intake as per above are allowed if blood Phe levels are < 30 µmol/L in order to achieve 2 x DRI guidelines (1.6 g/kg/day) of intact protein intake. This increased intake will now be the new reference baseline for dietary protein intake for future dietary assessments. Medical food may be discontinued once the dietitian determines that the essential amino acids meet the appropriate levels for age.

8.1.2.2 Dietary Changes for Blood Phe ≤360 µmol/L in Extension Phase (Subjects Randomized to Pegvaliase)

Once subjects initially randomized to active (pegvaliase) treatment enter the Extension Phase (Part 2) of the study, dietary protein intake may be modified if blood Phe concentration reaches ≤ 360 µmol/L for a minimum of 4 weeks. If diet is modified, it is recommended to increase protein from intact foods in 10 or 20 gram increments depending on intact protein intake, and decrease protein from medical food by a similar amount, 10 or 20 grams unless the subject is already consuming the appropriate protein levels for age (World Health Organization 2007). Medical food may be discontinued once the dietitian or investigator/designee determines that the essential amino acids provided from intact protein foods meet the appropriate levels for age.

From the beginning of the Extension Phase (Part 2), subjects initially randomized to active (pegvaliase) treatment who have achieved blood Phe > 30 to ≤360 µmol/L may have their medical food intake reduced or discontinued and intact protein intake increased, in

accordance with Table 8.1.2.2.1. Implementation of dietary adjustments will be performed in consultation with the site dietitian.

Table 8.1.2.2.1: Dietary Adjustment Algorithm in Part 2 for Subjects Randomized to Active (Pegvaliase) Treatment

Phe Category [a]	Protein Intake from Intact Food Sources [b]	Action	
		Protein Intake from Intact Food Sources	Protein Intake from Medical Food
> 30 [d] to ≤ 360 µmol/L	> 2 × RDI (> 1.7 g/kg)	Maintain or reduce by 10 g/day [c]	Discontinue [c]
	> 1 × to 2 × RDI (> 0.8 to 1.7 g/kg)	Maintain	Discontinue [c]
	0.5 × to 1 × RDI (0.4 to 0.8 g/kg)	Increase 10 g/day [c]	Decrease 10 g/day [c]
	< 0.5 × RDI (< 0.4 g/kg)	Increase 20 g/day [c]	Decrease 20 g/day [c]

RDI, recommended daily intake.

[a] Based on results of the previous blood Phe assessment; blood Phe reductions may not be observed until the subject is titrated to an effective dose.

[b] RDI (0.83 g/kg) is based on the joint FAO/WHO/UNU Expert Consultation on Protein and Amino Acid Requirements in Human Nutrition (World Health Organization 2007).

[c] The resulting adjusted dietary protein intake will become the new reference baseline for dietary protein intake for future dietary assessments.

[d] If a subject experiences blood Phe levels < 30 µmol/L on two consecutive blood tests, refer to the action to take in the event of blood Phe ≤ 30 µmol/L (Section 8.1.2.1).

8.1.3 Exploratory Assessments

8.1.3.1 Neurocognitive Assessments

The ADHD-RS IV and BRIEF instruments will be used to evaluate the effect of pegvaliase treatment on neurocognitive outcomes in adolescent subjects with PKU. The same parent/guardian must complete both the ADHD-RS IV and the BRIEF at all scheduled time points.

8.1.3.1.1 The ADHD Rating Scale IV Inattention Subscale (ADHDRS)

The ADHD-RS IV (American Psychiatric Association 2013; DuPaul 1998) will be used to assess inattention and hyperactivity symptoms among the adolescents enrolled in this study. The ADHD-RS is derived directly from diagnostic criteria delineated in the DSM, is well validated, and has been extensively used in a broad array of disease classes, including PKU (Gassio 2010; Wyrwich 2016). The ADHD-RS IV was the secondary endpoint in the

BioMarin Phase 3 pegvaliase study (165-302) in which improvement in inattention symptoms was associated with reduction in mean blood Phe levels (Thomas 2018).

The ADHD-RS IV instrument is completed independently by the parent/guardian. The same parent/guardian must complete the scale at each scheduled time point. The instrument consists of 2 nine-item scales that evaluate symptom severity for inattention and hyperactivity/impulsivity, respectively, during the previous month. Each scale is scored separately. Each item is rated on a 4-point severity scale (0, none; 1, mild; 2, moderate; 3, severe); scores of 2 or 3 on an individual item indicate a symptom. The PKU literature suggests that inattentive symptoms are more prevalent than hyperactivity and impulsivity symptoms among individuals with PKU; thus, the inattentive sub-scale is considered the most relevant and will be included in analyses (Bilder 2016).

A vendor specializing in cognitive testing provides study staff training for the investigator-rated ADHD-RS IV administration.

8.1.3.1.2 Behavior Rating Inventory of Executive Function (BRIEF)

The BRIEF is an 86-item measure designed to assess executive function skills in children 5 to 18 years of age (Gioia 2002). The BRIEF was developed to address the limitations of available assessments in examining real-word expressions of behaviors related to executive function. The BRIEF is intended to provide a standard method of asking multiple raters about executive function in daily life in a manner that is not specific to any particular disease. Because it is non-disorder-specific, BRIEF assesses executive function behaviors in children and adolescents experiencing a wide range of difficulties, such as those related to learning, attention, brain injuries, development disorders, and various psychiatric conditions and medical issues. The BRIEF is most often used to assess attention deficit hyperactivity disorder.

The BRIEF instrument (completed by the parent/guardian) contains 86 items in 8 non-overlapping clinical scales and 2 validity scales. The same parent/guardian must complete the BRIEF at each scheduled time point. These scales form 2 indexes: (1) Behavior Regulation (3 scales) and (2) Metacognition (5 scales), as well as a Global Executive Composite score which takes into account all of the clinical scales and represents the adolescent's overall executive function.

8.2 Safety Assessments

Planned time points for all safety assessments are provided in the SoA (Section 1.3).

8.2.1 Physical Examination

Physical examinations (full or brief) should be performed at the timepoints indicated in the SoA (Section 1.3):

- A full physical examination will include, at a minimum, general appearance (head, eyes, ears, nose, and throat) and an assessment of cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, musculoskeletal, and neurologic systems.
- A brief physical examination may be performed at the discretion of the investigator based on the subject's clinical condition and may include, but is not limited to, general appearance and an assessment of cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, musculoskeletal, and neurologic systems.

Investigators should pay special attention to clinical signs related to previous serious illnesses.

Height and weight will also be measured and recorded at the timepoints indicated in the SoA (Section 1.3).

8.2.2 Vital Signs

Vital signs include respiration rate, temperature, and single measurements of heart rate and seated diastolic and diastolic blood pressure. Any clinically significant change in vital signs will be recorded as an AE. Blood pressure and pulse measurements should be preceded by at least 5 minutes of rest for the subject in a quiet setting without distractions (eg, television, cell phones). Blood pressure and pulse measurements will be assessed with a completely automated device. Manual techniques will be used only if an automated device is not available.

Results will be recorded on the eCRF. Any abnormal vital sign assessments should be repeated once, and repeat value should be recorded in the eCRF.

8.2.3 Electrocardiograms

Electrocardiograms (ECGs) will be performed at Screening, Baseline, and SC/ET, as shown in the SoA (Section 1.3). Single 12-lead ECG will be obtained using an ECG machine that automatically calculates the heart rate and measures PR, QRS, QT, and QTc intervals.

8.2.4 Clinical Safety Laboratory Assessments

See Appendix 2, Section 10.2 for the table of clinical laboratory tests to be performed and refer to the SoA (Section 1.3) for the timing and frequency.

The investigator must review the laboratory report and document this review. The laboratory reports must be filed with the source documents. If known, the underlying diagnosis associated

with abnormal clinical laboratory test results that are considered clinically significant by the investigator should be recorded on the AE eCRF. Clinically significant abnormal laboratory findings are those which are not associated with the underlying disease, unless judged by the investigator to be more severe than expected for the subject's condition.

All abnormal laboratory tests with values considered clinically significant per the investigator during participation in the study and 30 days after the last dose of study treatment should be repeated until the values return to normal or baseline or are no longer considered clinically significant by the investigator.

In the event of elevated urinary protein on a test result, a repeat urinalysis should be performed. This repeat urine sample must be performed in the morning at the first or second morning void to allow for accurate test results and may be performed by a home healthcare nurse. Subjects with a confirmed urine/albumin creatinine ratio of ≥ 100 mg/g should be referred to a nephrologist for consultation if results were within normal range at Baseline. Subjects who had elevated results at Baseline followed by a confirmed subsequent increase of ≥ 100 mg/g from Baseline should also be referred to a nephrologist for consultation.

If clinically significant values do not return to normal/baseline within a period of time judged reasonable by the investigator, the etiology should be identified and the medical monitor notified.

All protocol-required laboratory assessments, as defined in Appendix 2, Section 10.2, must be conducted in accordance with the laboratory manual and the SoA.

If laboratory values from non-protocol specified laboratory assessments performed at the institution's local laboratory require a change in subject management or are considered clinically significant by the investigator (eg, SAE or AE or dose modification), then the results must be recorded in the eCRF.

8.2.5 Pregnancy Testing

Based on findings in studies of pregnant animals without PKU treated with pegvaliase-pqpz, pegvaliase may cause fetal harm when administered to a pregnant woman. Because the risks of taking study drug during pregnancy and breastfeeding in humans are unknown, subjects must not take study drug if they are trying to conceive, are pregnant, or are breastfeeding. From Day –28 until the end of the study, sexually active subjects must use 2 acceptable methods of contraception as outlined in the inclusion criteria section of this protocol (Section 5.1). Subjects who are confirmed to be pregnant by a serum pregnancy test and are temporarily off study drug are not required to perform the scheduled urine pregnancy tests.

In addition to contraception requirements, females of child-bearing potential are required to have urine pregnancy tests performed at the timepoints specified in the SoA (Section 5.1). Urine pregnancy test should be performed by the local laboratory. A serum pregnancy test will be performed by the central laboratory in the event of a positive or equivocal urine pregnancy test result.

Refer to Section 8.3.5 for details on the reporting procedures in the event of pregnancy.

8.3 Adverse Events and Serious Adverse Events

The definitions of an AE or SAE can be found in Appendix 3, Section 10.3.

AEs will be reported by the subject (or, when appropriate, by a caregiver, surrogate, or the subject's legally authorized representative).

The investigator and any medically qualified designees are responsible for identifying, documenting, and recording events that meet the definition of an AE or SAE and remain responsible for following up AEs that are serious, considered related to the study treatment or study procedures, or that caused the subject to discontinue the study treatment (refer to Section 7).

8.3.1 Time Period and Frequency for Collecting AE and SAE Information

Control (diet-only) treatment arm subject reporting:

- After informed consent to Day 1, only SAEs related to protocol mandated procedures will be collected.
- All AEs, SAEs, AESI, pregnancy and device related events will be collected from Day 1 through 4 weeks after last dose of study treatment or the study completion visit, whichever is longer.
- Non serious events that occur after informed consent but prior to Day 1 will be recorded on the Medical History/Concurrent Medical Conditions eCRF, not the AE eCRF.

Active (pegvaliase) treatment arm subject reporting:

- After informed consent through the first dose of study treatment, only SAEs related to protocol mandated procedures will be collected.
- All AEs, SAEs, AESI, pregnancy, and device-related events will be collected after the first dosed of study treatment through 4 weeks after the last dose of study treatment or the study completion visit, whichever is longer.
- Non serious events that begin after informed consent but before study treatment will be recorded on the Medical History/Concurrent Medical Conditions eCRF, not the AE eCRF.

All SAEs and AESIs must be recorded and reported to the sponsor or designee immediately; under no circumstances should this exceed 24 hours after the site becomes aware of the event, as indicated in Appendix 3, Section 10.3. The investigator must submit any updated SAE data to the sponsor within 24 hours of it being available.

Investigators are not obligated to actively seek AE or SAE information after conclusion of study participation. However, if the investigator learns of any SAE, including a death, at any time after a subject has been discharged from the study and he/she considers the event to be reasonably likely to be related to the study treatment or study participation, the investigator must promptly notify the sponsor.

8.3.2 Method of Identifying AEs and SAEs

The method of recording, evaluating, and assessing causality of AEs and SAEs and the procedures for completing and transmitting SAE reports are provided in Appendix 3, Section 10.3.

Care will be taken not to introduce bias when identifying AEs and/or SAEs. Open-ended and non-leading verbal questioning of the subject is the preferred method to inquire about AE occurrences.

8.3.3 Followup of AEs and SAEs

After the initial AE/SAE report, the investigator is required to proactively follow each subject at subsequent visits/contacts. All SAEs and AESIs (as defined in Section 8.3.8) will be followed until resolution, stabilization, the event is otherwise explained (ie, not related to study drug and/or study participation), or the subject is lost to follow-up (as defined in Section 7.3). Further information on follow-up procedures is provided in Appendix 3, Section 10.3.

8.3.4 Regulatory Reporting Requirements for SAEs

Prompt notification by the investigator to the sponsor of a SAE is essential so that legal obligations and ethical responsibilities towards the safety of subjects and the safety of a study treatment under clinical investigation can be met.

The sponsor has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a study treatment under clinical investigation. The sponsor will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority, Institutional Review Boards (IRB)/Independent Ethics Committees (IEC), and investigators.

Expedited safety reports must be prepared for suspected unexpected serious adverse reactions according to global regulatory requirements and sponsor policy and forwarded to investigators.

An investigator who receives an expedited safety report or other specific safety information from the sponsor will review and then file it along with the IB and will notify the IRB/IEC as appropriate.

8.3.5 Pregnancy

The importance of pregnancy prevention for study subjects and their partners will be communicated to all study subjects with regular reminders during the study.

Any female subject who becomes pregnant while participating in the study will temporarily discontinue study treatment.

Details of all pregnancies in female subjects and, if indicated, female partners of male subjects, will be collected after the start of study treatment and until 4 weeks after the last dose of study treatment.

While pregnancy itself is not considered to be an AE or an SAE, any pregnancy complication or elective termination of a pregnancy for medical reasons will be reported as an AE or SAE.

Abnormal pregnancy outcomes (eg, spontaneous abortion, fetal death, stillbirth, congenital anomalies, ectopic pregnancy), as well as any hospitalizations associated with pregnancy complications, unplanned caesarean section, and delivery complications should always be classified as an SAE (as the sponsor considers these to be medically significant), recorded on the eCRF, and expeditiously reported to the sponsor as an SAE following the procedures outlined in Appendix 3, Section 10.3.4.

The subject (or pregnant female partner) will be followed to determine the outcome of the pregnancy. The investigator will collect follow-up information on the subject (or pregnant female partner) and the neonate, and the information will be forwarded to the sponsor.

Any post-study pregnancy-related SAE considered reasonably related to the study treatment by the investigator should be reported to the sponsor as described in Section 8.3.4. While the investigator is not obligated to actively seek this information in former study subjects (or their pregnant female partners), he or she may learn of an SAE through spontaneous reporting.

Pregnancy reporting requirements to BioMarin are shown in Table 8.3.5.1.

Table 8.3.5.1: Pregnancy Reporting to BioMarin

Pregnancy Reporting to BioMarin
• Pregnancy and Pregnancy Outcome information will be entered into the electronic data collection system. • Pregnancy and Pregnancy Outcome information will be reported to BPV via the study specific Pregnancy Report and Pregnancy Outcome paper report forms. The site will complete the forms and submit via facsimile or email without 24 hours of awareness. • Abortion, Stillbirth, Fetal Congenital Anomaly and any other event meeting seriousness criteria must be reported as an SAE via the study electronic data collection system.

Male Subjects with Partners Who Become Pregnant

The investigator will attempt to collect pregnancy information on any male subject's female partner who becomes pregnant while the male subject is in this study. This applies only to male subjects who receive pegvaliase.

After obtaining the necessary signed informed consent from the pregnant female partner directly, the investigator will record pregnancy information on the appropriate form and submit it to the sponsor within 24 hours of learning of the partner's pregnancy. The female partner will also be followed to determine the outcome of the pregnancy. Information on the status of the mother and child will be forwarded to the sponsor. Generally, the follow-up will be no longer than 6 to 8 weeks following the estimated delivery date. Any termination of the pregnancy will be reported regardless of fetal status (presence or absence of anomalies) or indication for the procedure.

8.3.6 Cardiovascular and Death Events

All deaths that occur during the AE reporting period, regardless of attribution, will be recorded on the AE eCRF and expeditiously reported to the Sponsor as an SAE.

When recording a death, the event or condition that caused or contributed to the fatal outcome should be recorded as the single medical concept on the eCRF. If the cause of death is unknown and cannot be ascertained at the time of reporting, record "Unexplained Death" or "Death of Unknown Cause" on the eCRF.

8.3.7 DiseaseRelated Events and/or DiseaseRelated Outcomes Not Qualifying as AEs or SAEs

The following disease-related event (DRE) is common in subjects with PKU and can be serious:

- Hyperphenylalaninemia (Medical Dictionary for Regulatory Activities terminology [MedDRA] term: amino acid increased)

Because this event is typically associated with the disease under study, it will not be reported according to the standard process for expedited reporting of SAEs even though the event may meet the definition of a SAE. The elevated blood Phe levels should be recorded in the central laboratory database as per standard practice. These DREs will be monitored on a routine basis.

If either of the following conditions applies, then the event must be recorded and reported as an SAE (instead of a DRE):

- The event is, in the investigator's opinion, of greater intensity, frequency, or duration than expected for the individual subject; OR
- The investigator considers that there is a reasonable possibility that the event was related to study treatment.

8.3.8 Adverse Events of Special Interest

The following adverse events of special interest (AESI) must be reported to BPV within 24 hours of site awareness, irrespective of seriousness, severity, or causality to facilitate real-time monitoring and assessment. All AESI must be reported using the SAE form regardless of seriousness:

- Acute systemic hypersensitivity reaction, anaphylactic, or anaphylactoid reactions (refer to Appendix 5, Section 10.5)
- Angioedema
- Serum sickness
- Severe injection site reaction defined as an injection site reaction that is considered CTCAE Grade ≥ 3
- Hypophenylalaninemia events (defined as blood Phe < 30 µmol/L on 2 consecutive measurements)
- Arthralgia that is either persistent (AE duration > 6 months), severe (CTCAE Grade ≥ 3), or that requires treatment with a steroid (excluding topical)

8.3.8.1 Anaphylaxis (Acute Systemic Hypersensitivity Reaction)

All potential anaphylactic events, however reported, will be reviewed by BioMarin. When an event occurs which an investigator believes is possibly anaphylaxis, in addition to reporting the event to BPV within 24 hours of site awareness, it should be reported to the sponsor's medical monitor within 24 hours of site awareness.

In this protocol the term anaphylaxis is used to denote the important identified risk: acute systemic hypersensitivity reaction (see Section 2.3.1). An acute systemic hypersensitivity reaction (referred to as anaphylaxis in this protocol) is defined as an episode meeting National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network (NIAID/FAAN) criteria according to an internal BioMarin assessment performed by the Internal Data Safety Review Board. The Internal Safety Data Review Board, consisting of a representative from BioMarin clinical sciences, safety, and biostatistics departments, will review all cases of potential anaphylaxis to determine which of these events meet the criteria for an acute systemic hypersensitivity reaction as summarized in Appendix 5, Section 10.5.2.

In the clinical study report, analyses of anaphylaxis will include both the definition used in this protocol of an acute systemic hypersensitivity reaction and the FDA's definition of anaphylaxis, developed by the Division of Pulmonary, Allergy, and Rheumatology Products (DPARP) and used by BioMarin in the Palynziq US prescribing information (see the SAP for further details).

8.3.8.1.1 Individual Stopping Criteria Evaluation for Adverse Events During Pegvaliase Treatment

A subject who has an anaphylaxis episode that is suspected to meet Brown's criteria for severe (Grade 3) and that is, in the judgment of the investigator and/or the sponsor's medical monitor, related to study drug, will be permanently discontinued from study drug. Clinically severe hypersensitivity (Grade 3) per Brown's criteria is defined as significant hypoxia, hypotension or neurologic compromise that is life-threatening or required treatment to prevent a life-threatening event (Brown 2004):

- Cyanosis or $SpO_2 \leq 92\%$
- Hypotension with SBP < 90 mmHg
- Neurologic alteration: confusion, loss of consciousness, collapse, incontinence

In addition, a subject with a recurrent anaphylaxis episode of any severity that is, in the judgment of the investigator and/or the sponsor's medical monitor, related to study drug will be permanently discontinued from treatment.

8.3.8.1.2 Response to Anaphylaxis Episodes

If a subject experiences any symptoms suggestive of an anaphylaxis episode (as taught to the subject and adult observer in the initial and ongoing training on this risk), they will be instructed to inject epinephrine, to seek immediate medical attention, *and* to inform the investigator.

If the investigator suspects that an AE is anaphylaxis as defined by NIAID/FAAN, the subject will be assessed in the clinic and the sponsor's medical monitor must be immediately notified. Laboratory assessments for suspected anaphylaxis events described in the SoA (Section 1.3) under Hypersensitivity Reaction Visit (HRV) and in Table 6.5.1.1 should be performed prior to the next administration of study drug (if applicable) and should include anti-pegvaliase IgE (for optimal results, sampling should be performed 8 to 24 hours after event onset). If the investigator determines it is safe for the subject to resume dosing with study drug following resolution of anaphylaxis at any time during the study (excepting episodes requiring subject discontinuation, as outlined above), the following steps are required:

- At least the first dose administered after resolution of anaphylaxis is to be given at the clinic with equipment for emergency resuscitation (including epinephrine) within easy access.
- The subject must be premedicated with an H1 antagonist, and an H2 antagonist, and an antipyretic (eg, acetaminophen) approximately 2 to 3 hours prior to each dose of study drug for at least 1 week upon resumption of dosing, regardless of the duration of dose interruption.
- As with all doses of pegvaliase, an adult observer must observe the subject during study drug administration and for a minimum of 1 hour following study drug administration.

8.3.8.2 Angioedema

Angioedema is an eruption similar to urticaria but with larger, edematous wheals involving dermal, subcutaneous or submucosal tissues. It is sometimes associated with severe respiratory distress due to edema of the upper airways. Angioedema may present as a symptom (eg, lip, eye, tongue, throat, or facial swelling) of an acute systemic hypersensitivity reaction or anaphylaxis but may also present in isolation.

8.3.8.3 Serum Sickness

Serum sickness is a Type III immune hypersensitivity reaction that occurs when the immune system develops antibodies to a foreign protein. The antibody and foreign protein combine to form an immune complex that can settle in areas of the body such as the joints or the kidney.

Typical presentations include signs and symptoms commonly associated with hypersensitivity or infections: rash, itching, fever and arthralgia (MedDRA terms: serum sickness, serum sickness-like reaction and Type III immune-complex mediated reaction).

8.3.8.4 Severe Injection Site Reaction

A severe injection site reaction will be defined as any injection site reaction adverse event that is reported as CTCAE Grade 3 or above in severity and that codes to the MedDRA high level term (HLT) of Injection site reaction.

8.3.8.5 Hypophenylalaninemia

Blood Phe levels will be regularly monitored during the study. Hypophenylalaninemia is defined in this study as 2 consecutive blood Phe levels < 30 µmol/L. Hypophenylalaninemia will be managed in this study as described in Section 6.5.

8.3.8.6 Arthralgia

Arthralgia will be identified using the MedDRA PT of arthralgia and will include any synonyms of arthralgia such as musculoskeletal pain, pain in the extremity, or neck pain. Any event that meets these criteria and has a duration of > 6 months will be considered a persistent arthralgia. An arthralgia event that is reported as CTCAE Grade 3 or above in severity will be considered a severe event. Arthralgia events that require treatment with a steroid medication will also be considered an AESI.

8.3.9 Medical Device Incidents (Including Malfunctions)

Medical devices (prefilled syringes) are being provided for use in this study. In order to fulfill regulatory reporting obligations, the investigators are responsible for the detection and documentation of events meeting the definitions of device deficiency or malfunction that occur during the study with such devices.

The definition of a Medical Device deficiency can be found in Appendix 6, Section 10.6.

NOTE: Incidents fulfilling the definition of an AE/SAE will also follow the processes outlined in Section 8.3.3 and Appendix 3, Section 10.3.

8.3.9.1 Time Period for Detecting Medical Device Deficiencies

Medical device incidents or malfunctions of the device that result in an incident will be detected, documented, and reported during all periods of the study in which the medical device is used.

If the investigator learns of any device deficiency at any time after a subject has been discharged from the study, and such incident is considered reasonably related to a medical device provided for the study, the investigator will promptly notify the sponsor.

The method of documenting Medical Device deficiencies is provided in Appendix 6, Section 10.6.

8.3.9.2 Followup of Medical Device Deficiencies

Follow-up applies to all subjects, including those who discontinue study treatment.

The investigator is responsible for ensuring that follow-up includes any supplemental investigations as indicated to elucidate the nature and/or causality of the deficiency.

New or updated information will be recorded on the originally completed form with all changes signed and dated by the investigator.

8.3.9.3 Prompt Reporting of Device Deficiencies to Sponsor

Device deficiencies must be reported to the sponsor within 24 hours after the investigator determines that the event meets the protocol definition of a medical device deficiency.

The investigator should complete the Device Malfunction eCRF in electronic data capture (EDC). The investigator/designee must complete a Product Complaint form and submit it to the sponsor via fax or email as outlined in the Pharmacy Manual. The sponsor will be the contact for the receipt of device deficiency reports.

8.3.9.4 Regulatory Reporting Requirements for Device Deficiencies

The investigator must promptly report all device deficiencies occurring with any medical device provided for use in the study so the sponsor can fulfill their legal responsibility to notify appropriate regulatory authorities and other entities about certain safety information relating to medical devices being used in clinical studies.

The investigator, or responsible person according to local requirements (eg, the head of the medical institution), will comply with the applicable local regulatory requirements relating to the reporting of device deficiencies to the IRB/IEC.

8.4 Pharmacokinetics

Blood samples will be collected for measurement of plasma concentrations of pegvaliase as specified in the SoA (Section 1.3). For subjects randomized to active (pegvaliase) treatment, Part 1 blood samples for trough PK will be collected pre-dose (within 2 hours of dosing) at Day 1 and every 4 weeks for the first 24 weeks, followed by sampling every 8 weeks thereafter during Part 1. Intensive PK sampling will be performed at Week 73 and at dose

escalations to 20, 40, and 60 mg/day. For intensive PK, samples will be taken at pre-dose (within 2 hours of dosing) and 2, 4, 8, 12, and 24 hours post dose. The 24-hour sample will be taken prior to the next daily dose. For subjects randomized to control (diet-only) treatment, there are no PK assessments in Part 1.

For subjects randomized to active (pegvaliase) treatment, Extension Phase (Part 2) trough PK samples will be collected every 8 weeks. For subjects randomized to control (diet-only) treatment, the Part 2 PK assessment schedule will mirror the Part 1 schedule performed for active subjects, with trough PK samples collected pre-dose (within 2 hours of dosing) at Week 73 and every 4 weeks for the first 24 weeks and then every 8 weeks though the end of the study at Week 153. Intensive PK sampling will be performed Week 145 and for dose escalations to 20, 40, and 60 mg/day. For intensive PK, samples will be taken at pre-dose (within 2 hours of dosing) and 2, 4, 8, 12, and 24 hours post dose.

The PK parameters shown in Table 8.4.1 will be determined if feasible from the plasma concentration-time data for subjects who have sufficient data for PK analysis (eg, at least 3 samples with measurable concentrations during the terminal log-linear phase for estimating the terminal half-life and related parameters; at least 3 samples with measurable concentrations for other derived parameters).

Table 8.4.1: Derived Parameters for Pharmacokinetics Analysis

Parameter	Parameter Description
$AUC_{0-24hr,ss}$	Area under the plasma concentration-time curve from 0 to 24 hours at steady state, calculated using the linear trapezoidal rule
$C_{max,ss}$	Maximum observed plasma concentration within 24 hours post-dose at steady state
$T_{max,ss}$	Time to reach Cmax,ss
$C_{trough,ss}$	Trough observed plasma concentration prior to dosing at steady state
$t_{1/2}$	Terminal half-life, t1/2=ln2/λz where λz is the elimination rate constant
$CL_{ss/F}$	Apparent clearance of drug following subcutaneous injection at steady state
$V_{dz/F}$	Apparent volume of distribution based upon the terminal phase

The timing of sampling may be altered during the course of the study based on newly available data (eg, to obtain data closer to the time of peak plasma concentrations) to ensure appropriate monitoring. Instructions for the collection and handling of biological samples will be provided by the sponsor. The actual date and time (24-hour clock time) of each sample will be recorded.

Plasma samples collected for analyses of pegvaliase concentration may also be used for exploratory safety or efficacy analyses during or after the study.

Genetic analyses will not be performed on these plasma samples.

8.5 Pharmacodynamics

Blood Phe and Tyrosine (Tyr) will be measured by validated assays (see Section 8.1.1 and Section 1.3).

8.6 Biomarkers and Genetic Assessment

8.6.1 PAH Genotyping

PAH genotype testing should be performed where allowed by local health authorities and the EC/IRB.

8.6.2 Blood for Exploratory Analyses

As an exploratory analysis, blood will be collected to evaluate biochemical, molecular, and cellular aspects of PKU and to develop the assays used for these evaluations. The blood samples reserved for exploratory analyses are collected at the time points indicated in the SoA (see Section 1.3).

For each portion of blood samples reserved for protocol-specified analyses, there may be multiple sample aliquots. Once samples have been successfully analyzed during the study per protocol, unused sample portions may be used during the study and afterwards for assay development and other purposes stated in this section.

8.7 Immunogenicity Assessments

Sampling for immunogenicity testing will be performed routinely throughout the study to provide a comprehensive assessment of the immune response against pegvaliase. Antibodies against pegvaliase will be evaluated in serum samples collected from all subjects according to the SoA (Section 1.3). Additionally, serum samples should be collected at the final visit from subjects who discontinued study treatment or were withdrawn from the study.

Routine immunogenicity testing for anti-drug antibodies will be performed using validated semi-quantitative assays used in previous Phase 3 studies. Titer and incidence of positivity for each of the following analytes will be reported: total anti-pegvaliase antibodies (TAb), anti-PAL IgG, anti-PAL IgM, anti-PEG IgM, anti-PEG IgG, and pegvaliase-neutralizing antibodies (NAb). In the event of a hypersensitivity reaction visit (HRV) only, assessment of anti-pegvaliase IgE antibodies will be performed using an ImmunoCAP assay after depletion of IgG and IgM.

8.8 Health Economics/Medical Resource Utilization and Health Economics

Health Economics/Medical Resource Utilization and Health Economics parameters are not evaluated in this study.

9 STATISTICAL CONSIDERATIONS

9.1 Statistical Hypotheses

The study hypothesis is that subjects receiving pegvaliase will show a greater reduction in blood Phe following 72 weeks on study compared to the diet-only control group.

Let μ_a and μ_b denote the mean change from baseline in blood Phe following 72 weeks on study in the pegvaliase and diet-only group, respectively. The null and alternative hypotheses are:

H_0: $\mu_a = \mu_b$

H_1: $\mu_a \neq \mu_b$

The hypotheses will be tested at significance level of 0.05 (two-sided).

9.2 Sample Size Determination

Approximately 54 subjects will be enrolled into the study and randomized to the active (pegvaliase) or control (diet-only) group in 2:1 ratio. Assuming a mean (SD) reduction in Phe following 72 weeks on study of 640 (570) µmol/L in the pegvaliase group and 100 (250) µmol/L in the diet-only group, a total sample size of 54 subjects will provide more than 90% power to detect a treatment difference, based on the two-sample T-test with unequal variance and a significance level of 0.05 (two sided). The assumption of treatment effect is based on clinical data from previous pegvaliase studies and a PKU registry study. See the Statistical Analysis Plan (SAP) for additional details on the sample size calculation.

Approximately 72 subjects will be screened for study participation such that approximately 54 subjects will be enrolled in the study.

Note: Potential subjects who are screened for the purpose of determining eligibility for the study, but do not participate in the study, are not considered enrolled.

9.3 Populations for Analysis

The analysis populations are defined in Table 9.3.1.

Table 9.3.1: Analysis Populations

Population	Description
All Enrolled	All subjects (or their parent/guardians, if applicable) who agree to participate in the clinical study following completion of the informed consent process and determination of study eligibility.
ITT Population	All subjects who are randomized.
Safety Population	All subjects who received study treatment (pegvaliase or diet-only management of PKU).
Per Protocol Population	All subjects in the ITT population who are compliant with the protocol with no major protocol deviations impacting the efficacy analysis. Major protocol deviations impacting efficacy analysis will be defined and documented in the SAP.
PK Population	All subjects with at least one PK measurement

9.4 Statistical Analysis

The SAP will be finalized prior to database lock (DBL) and will include a more technical and detailed description of the statistical analyses described in this section. This section is a summary of the planned statistical analyses of the most important endpoints, including primary and key secondary endpoints.

A clinical pharmacology analysis plan will also be finalized prior to DBL.

Note: If discrepancies exist between the text of the statistical analysis as planned in the protocol, and the SAP, the SAP will prevail.

9.4.1 General Considerations

Descriptive analyses will be performed to gain an understanding of the qualitative and quantitative nature of the data collected and the characteristics of the sample collected. Continuous variables will be reported as mean, standard deviation (SD), median, minimum (min) and maximum (max). Categorical variables will be presented using absolute and relative frequency counts and percentage.

All generation of tables, listings and figures described in the SAP will be performed using SAS® version 9.4 or higher.

Full details on handling missing data and the corresponding analysis methods will be described in detail in the SAP.

9.4.2 Primary Endpoints

The primary efficacy endpoint is the change from baseline in blood Phe following 72 weeks on study. Baseline blood Phe is defined as the mean of 2 pre-treatment (Screening/Run-in)

measurements to be performed 7 to 10 days apart during the Screening/Run-in period. The blood Phe measurement following 72 weeks on study is defined as the average of the Week 69 and Week 73 blood Phe measurements.

Change from baseline will be compared between subjects in the active (pegvaliase) arm and the control (diet-only) arm. The primary efficacy endpoint will be evaluated using an Analysis of Covariance (ANCOVA) model, which will include baseline blood Phe as covariate and treatment group as factor, with missing data handled by multiple imputation method. The least square (LS) mean and 95% confidence interval will be calculated for the treatment difference between pegvaliase and diet-only arms following 72 weeks on study. Additional imputation method may be conducted as sensitivity analysis, further details will be provided in the SAP.

9.4.3 Secondary Endpoints

The secondary endpoints are as follows:

- Change from baseline in total dietary protein intake (ie, medical food and/or intact food). Baseline total protein intake is defined as the mean total dietary protein intake calculated from the 3 separate 3-day diet diaries collected during Screening/Run-in. The total dietary protein intake following 72 weeks on study is defined as the mean total dietary protein intake calculated from the diet diaries collected at the Week 69 and Week 73 visits.
- Plasma sample for trough PK

Descriptive summary will be provided. Full details will be provided in the SAP.

9.4.4 Exploratory Endpoints

Refer to the SAP for a discussion of analysis for exploratory endpoints.

9.4.5 Other Safety Analyses

Safety analyses will be performed on the Safety Population. AEs will be coded using the most recent version of the Medical Dictionary for Regulatory Activities (MedDRA). The incidence of treatment-emergent AEs, SAEs, and AESIs will be summarized by MedDRA system organ class, preferred term, relationship to study drug, and severity. Subjects with AEs that result in study drug discontinuation, dosing interruption, or dose level reduction will be tabulated. A by-subject listing of all AEs will be provided.

Clinical laboratory data will be summarized descriptively at baseline and post-baseline visits. Shift tables will be created to summarize the change in CTCAE grade from baseline to worst post-baseline value. Clinically significant laboratory abnormalities reported as AEs will be

summarized. Descriptive statistics for vital signs, physical examination results, ECG test results, and immunogenicity test results will also be provided. Details of statistical methods will be provided in the SAP.

9.5 Interim Analyses

No interim analyses are planned for this study.

9.6 Data Monitoring Committee (DMC)

The independent Data Monitoring Committee (DMC) will act in an advisory capacity to BioMarin to monitor subject safety and the efficacy of pegvaliase in subjects who participate in Study 165-306. The DMC responsibilities may include:

- Review the study protocol, informed consent and assent documents, and plans for data monitoring
- Evaluate the progress of the trial, study data quality, timeliness, subject recruitment, accrual and retention, subjects' risk versus benefit, and other factors that could affect the study outcome
- Consider relevant information that may have an impact on the safety of the subjects or the ethics of the study
- Protect the safety of the study subjects in accordance with the stopping rules as defined in study protocol
- Make recommendations to BioMarin concerning continuation, termination or other modifications of the study based on their observations of the study

Further details are provided in the DMC Charter.

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 Appendix 1: Regulatory, Ethical, and Study Oversight Considerations

10.1.1 Regulatory and Ethical Considerations

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

- Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines
- Principles of Good Clinical Practice as described in the US Code of Federal Regulations and the International Conference on Harmonisation Guidelines
- Applicable laws and regulations

The protocol, protocol amendments, ICF, Investigator Brochure, and other relevant documents (eg, advertisements) as applicable must be submitted, reviewed, and approved by the IRB/IEC before the study is initiated.

Any amendments to the protocol will require IRB/IEC approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study subjects.

The investigator will be responsible for the following:

- Providing written summaries of the status of the study to the IRB/IEC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/IEC
- Notifying the IRB/IEC of SAEs or other significant safety findings as required by IRB/IEC procedures
- Providing oversight of the conduct of the study at the site and adherence to requirements of 21 CFR, ICH guidelines, the IRB/IEC, European regulation 536/2014 for clinical studies (if applicable), and all other applicable local regulations

BioMarin will pay the full costs of the study-related tests, procedures, and treatments set forth in this protocol. In addition, after IRB/IEC approval, BioMarin may reimburse the reasonable cost of travel for study-related visits in accordance with BioMarin's travel and reimbursement policy.

The investigator should contact BioMarin immediately upon notification that a study subject has been injured by the study drug or by procedures performed as part of the study. Any subject who experiences a study-related injury should be instructed by the investigator to

seek immediate medical treatment at a pre-specified medical institution if possible, or at the closest medical treatment facility if necessary. The subject should be given the name of a person to contact to seek further information about, and assistance with, treatment for study-related injuries. BioMarin or the institution may pay for reasonable and necessary medical services to treat the injuries caused by the study drug or study procedures. In some jurisdictions, BioMarin is obligated by law to pay for study-related injuries. If this is the case, BioMarin will comply with the law. BioMarin will not pay for any hospitalizations, tests, or treatments for medical problems of any sort related solely to the study subject's primary disease or any concurrent disease and that are unrelated to the study.

Significant Noncompliance Reporting: Any deviation or series of deviations from the Protocol, ICH GCP, regulatory, and/or legal requirements that is likely to affect to a significant degree the safety or rights of a subject; the stability, quality, and/or control of study drug; or the quality or integrity of the study data shall be reported in writing to BioMarin within one (1) business day upon becoming aware of any suspicion or discovery of Significant Noncompliance.

10.1.2 Statement of Investigator and Financial Disclosure

Prior to beginning the study, the investigator at each site must provide to sponsor or designee a fully executed and signed Statement of Investigator (SOI) form. A US Food and Drug Administration (FDA) Form FDA 1572 serves as an acceptable SOI form. If Form FDA 1572 may not be used in a particular region, the investigator must provide a fully executed SOI on the form provided by the sponsor. All investigators and sub-investigators must be listed on Form FDA 1572 or its equivalent SOI. Financial Disclosure Forms must be completed with sufficient, accurate financial information as requested for all investigators and sub-investigators listed on the Form FDA 1572 or SOI who will be directly involved in the treatment or evaluation of subjects in this study. The Financial Disclosure Forms allow the sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities. Investigators must submit an updated Financial Disclosure Form to BioMarin should their response to any questions within the form or disclosure status change during the period of the study and for one year after study completion.

In accordance with FDA Form 1572 and/or principles of ICH GCP, the investigator will ensure that:

- He or 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 or she will personally conduct or supervise the study.
- He or 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 CFR Part 50 and/or ICH Sections 2.9 and 4.8 are met. As well, he or she will ensure that IRB/IEC review and approval in 21 CFR Part 56 and/or ICH Section 2.6 are met.
- He or she will report to the sponsor adverse experiences that occur in the course of the investigation in accordance with 21 CFR 312.64 and/or ICH Section 4.11.
- He or she has read and understands the information in the IB, including potential risks and side effects of the drug.
- His or her staff and all persons who assist in the conduct of the study are informed about their obligations in meeting the above commitments
- Adequate and accurate records in accordance with 21 CFR 312.62 and/or ICH Section 4.9 are kept, and those records are available for inspection in accordance with 21 CFR 312.68 and/or ICH Section 4.9.7.
- The IRB/EC/REB complies with the requirements of 21 CFR Part 56, ICH Section 3.0, and other applicable regulations, and conducts initial and ongoing reviews and approvals of the study. He or 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/REB. Additionally, he or she will not make any changes in the research without IRB/EC/REB approval, except where necessary to eliminate apparent immediate hazards to human subjects.
- He or she agrees to comply with all other requirements regarding the obligations of clinical Investigators and all other pertinent requirements in 21 CFR Part 312 and/or ICH E6 R2.

10.1.3 Informed Consent Process

The investigator or his/her representative will explain the nature of the study to the subject or his/her legally authorized representative and answer all questions regarding the study.

Subjects must be informed that their participation is voluntary. Subjects or their legally authorized representative (as determined by the laws of the jurisdiction in which the study is being conducted) will be required to sign a statement of informed consent that meets the regulatory requirements of the country or region where the subject consents, such as 21 CFR 50 (US FDA), ICH guidelines, privacy requirements including but not limited to the Health Insurance Portability and Accountability Act (HIPAA) requirements and the General Data Protection Regulation (where applicable), and the IRB/IEC or study center. If there is no applicable law addressing the issue of who may be a legally authorized representative, a legally authorized representative will be an individual recognized by institutional policy as

acceptable for providing consent on behalf of the prospective subject to the subject's participation in the procedure(s) involved in the research.

The medical record must include a statement that written informed consent was obtained before any study specific procedures were initiated and the date the written consent was obtained. The authorized person obtaining the informed consent must also sign the ICF.

Subjects must be re-consented to the most current version of the ICF(s) during their participation in the study. Subjects who reach the age of majority in their country while the study is ongoing will be asked to provide their own written consent again upon reaching the legal age of majority.

The ICF will contain a separate section that addresses the use of remaining samples for optional additional research. The investigator or authorized designee will explain to each subject the objectives of the additional research. Subjects will be told that they are free to refuse to participate and may withdraw their consent at any time and for any reason during the storage period. A separate signature will be required to document a subject's agreement to allow any remaining samples to be used for additional research.

A copy of the ICF(s) must be provided to the subject or the subject's legally authorized representative.

A subject who is rescreened is not required to sign another ICF if the rescreening occurs during the Screening/Run-in period.

10.1.4 Data Protection

Measures will be taken to ensure the privacy of subject data, including the use of subject numbers on eCRFs. A list linking subject identification numbers with subject names and other personal information will be kept in a secure place, separate from the subjects' medical records. BioMarin will follow all applicable regulations and guidelines in the relevant locality, country, and/or region to protect the privacy of subject data.

Subjects will be assigned a unique identifier by the sponsor. Any subject records or datasets that are transferred to the sponsor will contain the identifier only; subject names or any information which would make the subject identifiable will not be transferred.

The subject must be informed that his/her personal study-related data will be used by the sponsor in accordance with local data protection law. The level of disclosure must also be explained to the subject, who will be required to give consent or be given notice of the legal bases for their data to be used as described in the informed consent.

The subject must be informed that his/her medical records may be examined by authorized personnel appointed by the sponsor, by appropriate IRB/IEC members, and by inspectors from regulatory authorities.

10.1.5 Committee Structures

The following advisory and assurance activities will be implemented to support the study: monitoring committees, clinical trial disclosure, and study monitoring.

10.1.5.1 Data Monitoring Committee (DMC)

An external independent Data Monitoring Committee (DMC) will be responsible for monitoring subject safety and efficacy of pegvaliase. The DMC consisting of five members, who are each independent of the conduct of studies in the BioMarin BMN 165 (pegvaliase) development program, and are experts in the fields of PKU, clinical immunology, neuropsychology, pharmacovigilance, and biostatistics. DMC responsibilities are described in Section 9.6.

10.1.5.2 Internal Safety Data Review Board

The Internal Safety Data Review Board, consisting of a representative from BioMarin clinical sciences, safety, and biostatistics, will review all cases of potential anaphylaxis to determine which of these events meet the criteria for an acute systemic hypersensitivity reaction as summarized in Appendix 5, Section 10.5.2.

10.1.6 Dissemination of Clinical Study Data

The posting of study information and study results will comply with applicable national regulatory requirements and BioMarin's data sharing policy available at https://www.biomarin.com/data-request-form/.

10.1.7 Data Quality Assurance

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

Sites will enter study data into eCRFs in the study EDC system. Data Quality Control will be performed by BioMarin Clinical Data Management or a designee through implementation of quality control checks specified in the study data management plan and edit check specifications.

Study monitors will perform ongoing source data verification to confirm that data entered into the eCRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of subjects are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements. Qualified individuals designated by BioMarin will monitor all aspects of the study according to 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 subjects, and, if requested, agrees to assist the monitors. The investigator and staff are responsible for being present or available for consultation during routinely scheduled site visits conducted by BioMarin or its designees.

The investigator must retain all study records required by BioMarin and by the applicable regulations in a secure and safe facility. The investigator must notify BioMarin 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, that is, 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, subject 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) at least 2 years have elapsed since the formal discontinuation of clinical development of the IP. The investigator/institution should retain subject identifiers for at least 15 years after the completion or discontinuation of the study. Subject 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 a BioMarin agreement. BioMarin must be notified and will assist with retention should the investigator/institution be unable to continue maintenance of subject files for the full 15 years. It is the responsibility of BioMarin to inform the investigator/institution as to when these documents no longer need to be retained.

Representatives of BioMarin or designees may conduct an audit of a site at any time before, 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 inspection of the study. If informed of such an inspection, the investigator should notify BioMarin immediately. The investigator will ensure that the auditors/inspectors have access to the clinical supplies, site facilities, original source documentation, and all study files.

10.1.8 Source Documents

Source documents provide evidence for the existence of the subject and substantiate the integrity of the data collected. Source documents are filed at the investigator's site.

Data entered in the eCRF that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The investigator may need to request previous medical records or transfer records, depending on the study. Also, current medical records must be available.

The definition of what constitutes source data can be found in the Monitoring Plan.

10.1.9 Study and Site Start and Closure

First Act of Recruitment

The study start date is the date on which the clinical study will be open for recruitment of subjects.

The first act of recruitment is the first site open and will be the study start date.

Study/Site Termination

The sponsor reserves the right to close the site or terminate the study at any time for any reason at the sole discretion of the sponsor. Sites will be closed upon study completion. A site is considered closed when all required documents and unused study supplies have been collected and a site closure visit has been performed.

Reasons for the early closure of a site by the sponsor may include but are not limited to:

- Failure of the investigator to comply with the protocol, the requirements of the IRB/IEC or local health authorities, the sponsor's procedures, or GCP guidelines
- Inadequate recruitment of subjects by the investigator
- Discontinuation of further study treatment development

If the study is prematurely terminated or suspended, the sponsor shall promptly inform the investigators, the IECs/IRBs, the regulatory authorities, and any contract research organization(s) used in the study of the reason for termination or suspension, as specified by the applicable regulatory requirements. The investigator shall promptly inform the subject(s) and should assure appropriate subject therapy and/or follow-up.

10.1.10 Publication Policy

The results of this study may be published or presented at scientific meetings. If this is foreseen, the investigators agree to submit all manuscripts or abstracts to the sponsor at least forty-five (45) days before submission or as agreed in the Clinical Trial Agreement between BioMarin and the investigator/institution. This allows the sponsor to protect proprietary information and to provide comments.

The sponsor will comply with the requirements for publication of study results. In accordance with standard editorial and ethical practice, the sponsor will generally support publication of multicenter studies only in their entirety and not as individual site data. In this case, a coordinating investigator will be designated by mutual agreement.

BioMarin recognizes the importance of communicating medical study data and therefore encourages the publication of these data 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 may be described in the Clinical Trial Agreement between BioMarin and the investigator/institution. Consideration for authorship of all publications will be based on mutual agreement and compliance with the Uniform Requirements for Manuscripts Submitted to Biomedical Journals (“Uniform Requirements”) of the International Committee of Medical Journal Editors (ICMJE) (http://www.icmje.org/about-icmje/faqs/icmje-recommendations/) and good publication practices (GPP).

10.2 Appendix 2: Clinical Laboratory Tests

The tests detailed in Table 10.2.1 will be performed by the central laboratory.

Protocol-specific requirements for inclusion or exclusion of subjects are detailed in Section 5 of the protocol.

Additional tests may be performed at any time during the study as determined necessary by the investigator or required by local regulations.

Table 10.2.1: Clinical Laboratory Tests

Laboratory Test	Parameters		
Chemistry	Albumin Alkaline Phosphatase ALT (SGPT) AST (SGOT) Bicarbonate Direct bilirubin Total bilirubin BUN	Calcium Total cholesterol CO_2 Creatinine Creatine phosphokinase Glucose (fasting) GGT	LDH Phosphorus Potassium Total protein Sodium Uric acid
Hematology	Hemoglobin Hematocrit	WBC count RBC count	Platelet count Differential cell count
Urinalysis	Appearance Color pH Specific gravity Ketone Protein	Glucose Bilirubin Nitrite Urobilinogen Hemoglobin	Urinary albumin/creatinine ratio [a] Urinary microscopy Urinary N-methyl histamine
Pregnancy testing	Refer to Section 8.2.5		
Complement panel	C3 C4		
Other tests conducted during the study (refer to the SoA for timing)	Phenylalanine Tyrosine hs-CRP Serum tryptase level Serum cortisol [b] Erythrocyte sedimentation rate [c]		

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; C3, C4, complement components 3, 4; CO2, carbon dioxide; ESR, Erythrocyte sedimentation rate; GGT, gamma-glutamyltransferase; hs-CRP, high sensitivity C-reactive protein; LDH, lactate dehydrogenase; RBC, red blood cell; SGOT, serum glutamic-oxaloacetic transaminase; SGPT, serum glutamic-pyruvic transaminase; WBC, white blood cell.

[a] It is recommended that urine samples be obtained as a first or second morning void. Subjects with a confirmed urine/albumin creatinine ratio of ≥ 100 mg/g should be referred to a nephrologist for consultation if results were within normal range at Baseline. Subjects who had elevated results at Baseline followed by a confirmed subsequent increase of 100-200 mg/g from Baseline should also be referred to a nephrologist for consultation.

[b] Serum cortisol samples should be taken before study drug administration in the morning before 10:00 am. A home healthcare nurse may collect samples for subject convenience. If 2 results are low and abnormal, the subject will be asked to perform additional sampling for plasma ACTH and a low-dose conventional ACTH stimulation test or the ACTH test method based on the clinical practice at the site.

[c] ESR will be performed at a local laboratory.

Investigators must document their review of each laboratory safety report.

10.3 Appendix 3: Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting

10.3.1 Definition of an Adverse Event

AE Definition
• An AE is any untoward medical occurrence in a patient or clinical study subject, temporally associated with the use of study treatment, whether or not considered related to the study treatment. • NOTE: An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of study treatment.

Events Meeting the AE Definition
• Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (eg, ECG, radiological scans, vital signs measurements), including those that worsen from baseline, considered clinically significant in the medical and scientific judgment of the investigator (ie, not related to progression of underlying disease). • Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition. • New conditions detected or diagnosed after study treatment administration • Signs, symptoms, or the clinical sequelae of a suspected drug-drug interaction. • Signs, symptoms, or the clinical sequelae of a suspected overdose of either study treatment or a concomitant medication. Overdose per se will not be reported as an AE/SAE unless it is an intentional overdose taken with possible suicidal/self-harming intent. Such overdoses should be reported regardless of sequelae. • "Lack of efficacy" or "failure of expected pharmacological action" per se will not be reported as an AE or SAE. Such instances will be captured in the efficacy assessments. However, the signs, symptoms, and/or clinical sequelae resulting from lack of efficacy will be reported as AE or SAE if they fulfill the definition of an AE or SAE.

Events Not Meeting the AE Definition
• Any clinically significant abnormal laboratory findings or other abnormal safety assessments which are associated with the underlying disease, unless judged by the investigator to be more severe than expected for the subject's condition. • The disease/disorder being studied or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the subject's condition. • Medical or surgical procedure (eg, endoscopy, appendectomy): the condition that leads to the procedure is the AE. • Situations in which an untoward medical occurrence did not occur (social and/or convenience admission to a hospital). • Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.

Events Not Meeting the AE Definition
• Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline • Planned hospitalization for childbirth (eg, scheduled Caesarean section)

10.3.2 Definition of an SAE

If an event is not an AE per the definition above, then it cannot be an SAE even if serious conditions are met (eg, hospitalization for signs/symptoms of the disease under study, death due to progression of disease).

A SAE is defined as any untoward medical occurrence that, at any dose:
a. Results in death
b. Is life-threatening The term "life-threatening" in the definition of "serious" refers to an event in which the subject was at risk of death at the time of the event. It does not refer to an event that hypothetically might have caused death if it were more severe.
c. Requires inpatient hospitalization or prolongation of existing hospitalization In general, hospitalization signifies that the subject has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious.
d. Results in persistent disability/incapacity The term disability means a substantial disruption of a person's ability to conduct normal life functions. This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (eg, sprained ankle) which may interfere with or prevent everyday life functions but do not constitute a substantial disruption.
e. Is a congenital anomaly/birth defect in the offspring of a study subject
f. Other situations/Important Medical Events: Medical or scientific judgment should be exercised in deciding whether SAE reporting is appropriate in other situations such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require medical or surgical treatment to prevent one of the other outcomes listed in the above definition. These events should usually be considered serious. Examples of such events include invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

10.3.3 Recording and Follow-Up of AEs and/or SAEs

AE and SAE Recording
• When an AE/SAE occurs, it is the responsibility of the investigator to review all documentation (eg, hospital progress notes, laboratory reports, and diagnostics reports) related to the event. • The investigator or qualified designee will then record all relevant AE/SAE information in the eCRF. • It is **not** acceptable for the investigator to send photocopies of the subject's medical records to BioMarin in lieu of completion of the AE/SAE eCRF page. • There may be instances when copies of medical records for certain cases are requested by BioMarin. In these cases, all subject identifiers, with the exception of the subject number, will be redacted on the copies of the medical records before submission to BioMarin. • The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. Whenever possible, the diagnosis (not the individual signs/symptoms) will be documented as the AE/SAE.
Assessment of Intensity
The investigator will make an assessment of intensity for each AE and SAE reported during the study and assign it to 1 of the following categories (as defined in Common Terminology Criteria for Adverse Events [CTCAE] version 5.0). The investigator should only record the single highest grade of intensity for each AE and SAE in the eCRF. Adverse events that do not have a corresponding CTCAE term will be assessed according to the general guidelines for grading: • Grade 1 (Mild): asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated • Grade 2: (Moderate): minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL) (eg, preparing meals, shopping for groceries or clothes, using the telephone, managing money) • Grade 3 (Severe): medically significant but not immediately life-threatening: hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL (eg, bathing, dressing/undressing, feeding oneself, using the toilet, taking medications, not bedridden) • Grade 4: Life threatening consequences; urgent intervention indicated • Grade 5: Death related to AE An event is defined as "serious" when it meets at least 1 of the predefined outcomes as described in the definition of an SAE, NOT when it is rated as severe.

Assessment of Causality
• The investigator is obligated to assess the relationship between study treatment and each occurrence of each AE/SAE. • An assessment of "related" means that there are facts, evidence, and/or arguments to suggest a causal relationship such that a relationship cannot be ruled out. • The investigator will use clinical judgment to determine the relationship. • Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to study treatment administration will be considered and investigated.

Assessment of Causality
• For each AE/SAE, the investigator must document in the medical notes that he/she has reviewed the AE/SAE and has provided an assessment of causality. • There may be situations in which an SAE has occurred, and the investigator has minimal information to include in the initial report to BioMarin. However, it is very important that the investigator always make an assessment of causality for every event before the initial transmission of the SAE data to BioMarin. • The investigator may change his/her opinion of causality in light of follow-up information and send a SAE follow-up report with the updated causality assessment.

Follow-up of AEs and SAEs
• The investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by BioMarin to elucidate the nature and/or causality of the AE or SAE as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals. • If a subject dies during participation in the study or during a recognized follow-up period, the investigator will provide a copy of any post-mortem findings including histopathology. • New or updated information will be recorded in the originally completed eCRF. • The investigator will submit any updated SAE data to the sponsor within 24 hours of receipt of the information.

10.3.4 Reporting of SAEs and Events of Special Interest

SAE/AESI Reporting to BioMarin Pharmacovigilance (BPV) via an Electronic Data Collection Tool
• The primary mechanism for reporting an SAE/AESI to BPV will be the electronic data collection tool. AESI reporting will follow the SAE reporting requirements. • If the electronic system is unavailable, then the site will use the paper SAE data collection tool (see next section) in order to report the event within 24 hours. • The site will enter the SAE/AESI data into the electronic system as soon as it becomes available. • After the study is completed at a given site, the electronic data collection tool will be taken off-line to prevent the entry of new data or changes to existing data. • If a site receives a report of a new SAE/AESI from a study subject or receives updated data on a previously reported SAE/AESI after the electronic data collection tool has been taken off-line, then the site can report this information on a paper SAE form (see next section) or to BPV by telephone.

SAE/AESI Reporting to BPV via Paper CRF
• Facsimile transmission or email of the paper CRF (pregnancy form, pregnancy outcome, or SAE form is EDC unavailable) is the preferred method to transmit this information to BPV. • In rare circumstances and in the absence of facsimile equipment, notification by telephone is acceptable with a copy of the paper CRF sent by overnight mail or courier service. • Initial notification via telephone does not replace the need for the investigator to complete and sign the CRF pages within the designated reporting time frames.

Contact information for BioMarin Pharmacovigilance is as follows:

BioMarin Pharmaceutical Inc.
Address 105 Digital Drive
Novato, CA 94949
Phone: PI
Fax:
E-mail: drugsafety@bmrn.com

The Investigator is encouraged to discuss with the medical monitor any AEs for which the issue of seriousness is unclear or questioned. Contact information for the medical monitor is provided on the signature page at the front of this protocol.

Name: PI RN, MS
Address: 105 Digital Drive
Novato, CA 94949 USA
Phone: PI
Fax:
E-mail:

10.4 Appendix 4: Contraceptive and Barrier Guidance

Definitions:

Woman of Childbearing Potential (WOCBP)

A woman is considered fertile following menarche and until becoming post-menopausal unless permanently sterile (see below).

If fertility is unclear (eg, amenorrhea in adolescents or athletes) and a menstrual cycle cannot be confirmed before first dose of study treatment, additional evaluation should be considered.

Women in the following categories are not considered WOCBP:

- Premenarchal
- Premenopausal female with 1 of the following:
    - Documented hysterectomy
    - Documented bilateral salpingectomy
    - Documented bilateral oophorectomy

  For individuals with permanent infertility due to a medical cause other than the above (eg, mullerian agenesis, androgen insensitivity), investigator discretion should be applied in determining study eligibility.

  Note: Documentation can come from the site personnel's review of the subject's medical records, medical examination, or medical history interview.
- Postmenopausal female
    - A postmenopausal state is defined as no menses for 12 months without an alternative medical cause.
        - A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a postmenopausal state in women not using hormonal contraception or hormonal replacement therapy (HRT). However, in the absence of 12 months of amenorrhea, confirmation with more than one FSH measurement is required.
    - Females on HRT whose menopausal status is in doubt will be required to use one of the non-estrogen hormonal highly effective contraception methods if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of postmenopausal status before study enrollment.

Contraception Guidance:

If sexually active, must be willing to use two acceptable methods of contraception while participating in the study and 4 weeks after the last dose of pegvaliase.

10.5 Appendix 5: Anaphylaxis Criteria

For the purposes of this study, the term anaphylaxis refers to the important identified risk of acute systemic hypersensitivity reaction. An acute systemic hypersensitivity reaction is one that is consistent with the NIAID/FAAN definition as assessed by BioMarin using the criteria listed below in Appendix 5, Section 10.5.1 and Appendix 5, Section 10.5.2.

10.5.1 Sampson's Anaphylaxis Criteria

According to the National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network (NIAID/FAAN) Second Symposium on the definition and management of anaphylaxis, anaphylaxis is highly likely when any one of the following 3 criteria are fulfilled:

1. Acute onset of an illness (minutes to several hours) with involvement of the skin, mucosal tissue, or both (eg, generalized hives, pruritus or flushing, swollen lipstongueuvula)

   AND AT LEAST ONE OF THE FOLLOWING:

   a. Respiratory compromise (eg, dyspnea, wheezebronchospasm, stridor, reduced peak expiratory flow [PEF], hypoxemia)

   b. Reduced blood pressure (BP) or associated symptoms of endorgan dysfunction (eg, hypotonia [collapse], syncope, incontinence)

2. Two or more of the following that occur rapidly after exposure *to a likely allergen for that patient* (minutes to several hours):

   a. Involvement of the skinmucosal tissue (eg, generalized hives, itchflush, swollen lipstongueuvula)

   b. Respiratory compromise (eg, dyspnea, wheezebronchospasm, stridor, reduced PEF, hypoxemia)

   c. Reduced BP or associated symptoms (eg, hypotonia [collapse], syncope, incontinence)

   d. Persistent gastrointestinal symptoms (eg, crampy abdominal pain, vomiting)

3. Reduced BP after exposure to *known allergen for that patient* (minutes to several hours):

a. Infants and children: low systolic blood pressure (age specific) or greater than 30% decrease is systolic BP

b. Adults: systolic BP of less than 90 mmHg or greater than 30% decrease from that person's baseline.

Source: Sampson 2006.

10.5.2 Acute Systemic Hypersensitivity Reaction

An acute systemic hypersensitivity reaction is a Type III acute systemic allergic reaction. An acute systemic hypersensitivity reaction has a similar clinical presentation to an anaphylactic reaction, but only meets the definition if the italicized criteria below are met in addition to the criteria outlined in Sampson's criteria (see Appendix 5, Section 10.5.1, Sampson's Criteria):

Sampson Criteria Number	Sampson Criteria Wording With Additional Criteria Necessary to Meet Acute Systemic Hypersensitivity Reaction Criteria Shown in Italics
1	Acute onset of an illness (minutes to several hours) with involvement of the skin, mucosal tissue, or both (eg, generalized hives, pruritus or flushing, swollen lips-tongue-uvula). *Must be generalized skin reaction to meet criteria*.
1a	Respiratory compromise (eg, dyspnea, wheeze-bronchospasm, stridor, reduced PEF, hypoxemia). *Respiratory compromise is demonstrated by oxygen desaturation below 88% on room air.*
1b	Reduced BP or associated symptoms of end-organ dysfunction (eg, hypotonia [collapse], syncope, incontinence). *Systolic BP less than 90 mmHg (adults) or 70 mmHg (children) or greater than 30% decrease from baseline*.
2d	Persistent gastrointestinal symptoms (eg, crampy abdominal pain, vomiting). *Gastrointestinal symptoms are persistent with multiple episodes that continue for several hours or more*.

10.5.3 Anaphylaxis Analysis

During the initial BLA for Palynziq in the US, the FDA proposed that anaphylaxis should be defined for labelling purposes using the methodology developed by FDA's Division of Pulmonary, Allergy, and Rheumatology Products (DPARP). As a result, in the study analyses for 165-306 anaphylaxis, including derivation of the incidence and event rates, data will be presented using both BioMarin's definition of an acute systemic hypersensitivity reaction described above and the FDA's assessment methodology, ie, a case will be counted as an anaphylaxis event if it meets any of the following criteria:

- Clinical manifestations consistent with NIAID/FAAN criterion #1 (skin involvement + respiratory or cardiovascular compromise); or

- Verbatim reports of anaphylaxis/anaphylactic reaction (or synonyms), even if NIAID/FAAN criteria were not met; or
- Cases involving subject injection of epinephrine alone, even if the subject did not experience signs/symptoms necessary to meet NIAID/FAAN criteria.

Further details are provided in the SAP.

10.6 Appendix 6: Medical Device Adverse Events, Adverse Device Effects (ADEs), Serious Adverse Events (SAEs), and Device Deficiencies: Definition and Procedures for Recording, Evaluating, Follow-Up, and Reporting

Medical Device Adverse Event reporting applies only to US sites. The definitions and procedures detailed in this appendix are in accordance with ISO 14155.

Both the investigator and the sponsor will comply with all local medical device reporting requirements.

The detection and documentation procedures described in this protocol apply to all sponsor medical devices provided for use in the study. For this study, prefilled syringes are provided (see Section 6.1.1).

10.6.1 Definition of AE and ADE

AE and ADE Definition
• An AE is defined as any untoward medical occurrence, unintended disease or injury, or untoward clinical signs (including abnormal laboratory finding) in study subjects, users, or other persons, whether or not related to the investigational medical device. This definition includes events related to the investigational medical device or comparator and events related to the procedures involved except for events in users or other persons, which only include events related to investigational devices. • An adverse device effect (ADE) is defined as an adverse event related to the use of an investigational medical device. This definition includes any adverse events resulting from insufficient or inadequate instructions for use, deployment, implantation, installation, or operation, or any malfunction of the investigational medical device as well as any event resulting from use error or from intentional misuse of the investigational medical device.

10.6.2 Definition of SAE, SADE, and USADE

If an event is not an AE per definition above, then it cannot be an SAE even if serious conditions are met (eg, hospitalization for signs/symptoms of the disease under study, death due to disease progression).

An SAE is an AE that:
• Led to death • Led to serious deterioration in the health of the subject resulting in either: 1. A life-threatening illness or injury. The term 'life-threatening' in the definition of 'serious' refers to an event in which the subject was at risk of death at the time of the event. It does not refer to an event which hypothetically might have caused death if it were more severe. 2. A permanent impairment of a body structure or a body function 3. Inpatient or prolonged hospitalization, planned hospitalization for a pre-existing condition, or a procedure required by the protocol, without serious deterioration in health, is not considered an SAE. 4. Medical or surgical intervention to prevent life-threatening illness or injury or permanent impairment to a body structure or a body function • Led to fetal distress, fetal death, or a congenital abnormality or birth defect
SADE definition:
• A SADE is defined as an adverse device effect that has resulted in any of the consequences characteristic of a serious adverse event.
USADE definition
• A USADE is a serious adverse device effect which by its nature, incidence, severity, or outcome has not been identified in the current version of the risk analysis report.

10.6.3 Definition of Device Deficiency

Device deficiency definition
• A device deficiency is an inadequacy of a medical device with respect to its identity, quality, durability, reliability, safety, or performance. Device deficiencies include malfunctions, use errors, and inadequate labeling.

10.6.4 Recording and FollowUp of AE and/or SAE and Device Deficiencies

AE, SAE, and Device Deficiency Recording
• When an AE/SAE/device deficiency occurs, it is the responsibility of the investigator to review all documentation (e.g., hospital progress notes, laboratory reports, and diagnostics reports) related to the event. • The investigator will then record all relevant AE/SAE/device deficiency information in the subject's medical records, in accordance with the investigator's normal clinical practice and on the appropriate form of the eCRF. • All ADEs will be entered into the AE eCRF. • SADEs will be entered into the AE eCRF and submitted to BPV. The site will also complete and submit the study specific paper device report form within 24 hours of site awareness. • It is not acceptable for the investigator to send photocopies of the subject's medical records to BPV in lieu of completion of the /AE/SAE/device deficiency eCRF page. • There may be instances when copies of medical records for certain cases are requested by BPV. In this case, all subject identifiers, with the exception of the subject number, will be redacted on the copies of the medical records before submission to BPV. • The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. Whenever possible, the diagnosis (not the individual signs/symptoms) will be documented as the AE/SAE. • For device deficiencies, it is very important that the investigator describes any corrective or remedial actions taken to prevent recurrence of the incident. • A remedial action is any action other than routine maintenance or servicing of a medical device where such action is necessary to prevent recurrence of a device deficiency. This includes any amendment to the device design to prevent recurrence.
Assessment of Intensity

• The investigator will make an assessment of intensity for each AE/SAE/device deficiency reported during the study and assign it to one of the following categories:
  ○ Mild: An event that is easily tolerated by the subject, causing minimal discomfort and not interfering with everyday activities.
  ○ Moderate: An event that causes sufficient discomfort and interferes with normal everyday activities.
  ○ Severe: An event that prevents normal everyday activities. An AE that is assessed as severe should not be confused with a SAE. Severe is a category utilized for rating the intensity of an event; and both AEs and SAEs can be assessed as severe.
• An event is defined as 'serious' when it meets at least 1 of the predefined outcomes as described in the definition of an SAE, NOT when it is rated as severe.

Assessment of Causality
• The investigator is obligated to assess the relationship between study treatment and each occurrence of each AE/SAE/device deficiency. • A "reasonable possibility" of a relationship conveys that there are facts, evidence, and/or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out. • The investigator will use clinical judgment to determine the relationship. • Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to study treatment administration will be considered and investigated. • The investigator will also consult the IB in his/her assessment. • For each AE/SAE/device deficiency, the investigator must document in the medical notes that he/she has reviewed the AE/SAE/device deficiency and has provided an assessment of causality. • There may be situations in which an SAE has occurred and the investigator has minimal information to include in the initial report to BPV. However, it is very important that the investigator always make an assessment of causality for every event before the initial transmission of the SAE data to BPV. • The investigator may change his/her opinion of causality in light of follow-up information and send a SAE follow-up report with the updated causality assessment. • The causality assessment is one of the criteria used when determining regulatory reporting requirements.

Follow-up of AE/SAE/device deficiency
• The investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by BPV to elucidate the nature and/or causality of the AE/SAE/device deficiency as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals. • If a subject dies during participation in the study or during a recognized follow-up period, the investigator will provide BPV with a copy of any post mortem findings including histopathology. • New or updated information will be recorded in the originally completed eCRF. • The investigator will submit any updated SAE data to BPV within 24 hours of receipt of the information.

10.6.5 Reporting of SAEs

SAE Reporting to BPV via an Electronic Data Collection Tool
• The primary mechanism for reporting an SAE to BPV will be the electronic data collection tool. • If the electronic system is unavailable, then the site will use the paper SAE data collection tool (see next section) in order to report the event within 24 hours. • The site will enter the SAE data into the electronic system as soon as it becomes available. • After the study is completed at a given site, the electronic data collection tool will be taken off-line to prevent the entry of new data or changes to existing data. • If a site receives a report of a new SAE from a study subject or receives updated data on a previously reported SAE after the electronic data collection tool has been taken off-line, then the site can report this information on a paper SAE form (see next section) or to BPV by telephone. • Contacts for SAE reporting can be found in Appendix 3, Section 10.3.4.

SAE Reporting to BPV via Paper CRF
• Facsimile transmission of the SAE paper CRF is the preferred method to transmit this information to BPV. • In rare circumstances and in the absence of facsimile equipment, notification by telephone is acceptable with a copy of the SAE data collection tool sent by overnight mail or courier service. • Initial notification via telephone does not replace the need for the investigator to complete and sign the SAE CRF pages within the designated reporting time frames. • Contacts for SAE reporting can be found in Appendix 3, Section 10.3.4.

10.6.6 Reporting of SADEs

SAE Reporting to BPV
NOTE: There are additional reporting obligations for medical device incidents that are potentially related to SAEs that must fulfil the legal responsibility to notify appropriate regulatory authorities and other entities about certain safety information relating to medical devices being used in clinical studies. • Any device deficiency that is associated with an SAE must be reported to the sponsor within 24 hours after the investigator determines that the event meets the definition of a device deficiency. • The sponsor shall review all device deficiencies and determine and document in writing whether they could have led to an SAE. These shall be reported to the regulatory authorities and IRBs/IECs as required by national regulations. • Contacts for SAE reporting can be found in Appendix 3, Section 10.3.4.

10.7 Appendix 7: Abbreviations

Abbreviation	Definition
ACMG	American College of Medical Genetics and Genomics
ADHD	Attention Deficit Hyperactivity Disorder
ADHD-RS	Attention Deficit Hyperactivity Disorder Rating Scale
ADL	activities of daily living
AE	adverse event
AESI	adverse events of special interest
ALT	alanine aminotransferase
ANCOVA	Analysis of Covariance
AST	aspartate aminotransferase
AUC	area under the concentration-time curve
BH4	tetrahydrobiopterin
BLA	Biologics License Application
BPV	BioMarin Pharmacovigilance
BRIEF	Behavior Rating Inventory of Executive Function
C3	complement component 3
C4	complement component 4
CIOMS	Council for International Organizations of Medical Sciences
C_{max}	maximum observed plasma concentration
CONSORT	Consolidated Standards of Reporting Trials
CRF	case report form
CRP	C-reactive protein
CTCAE	Common Terminology Criteria for Adverse Events
DBL	database lock
DFRF	Directed Follow-up Report Form
DMC	Data Monitoring Committee
DPARP	FDA Division of Pulmonary, Allergy, and Rheumatology Products
DRE	disease-related event
DRI	Dietary Reference Intake
DSM	Diagnostic and Statistical Manual of Mental Disorders
ECG	electrocardiogram
EC/IRB	Ethics Committee/Institutional Review Board
eCRF	electronic Case Report Form
EDC	electronic data capture

Abbreviation	Definition
ET	Early Termination
EU	European Union
FDA	Food and Drug Administration
FSH	follicle stimulating hormone
GCP	good clinical practice
GPP	good publication practice
HAE	hypersensitivity adverse event
HIPAA	Health Insurance Portability and Accountability Act
HPA	hyperphenylalaninemia
HRT	hormonal replacement therapy
HRV	hypersensitivity reaction visit
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICMJE	International Committee of Medical Journal Editors
IgE	immunoglobulin E
IgG	immunoglobulin G
IgM	immunoglobulin M
IMP	investigational medical products
IND	investigational new drug
IP	investigational product
IQ	intelligence quotient
ISR	injection site reaction
I/T/M	Induction/Titration/Maintenance (dosing regimen)
LS	least square
MedDRA	Medical Dictionary for Regulatory Activities
MNT	medical nutritional therapy
NAb	neutralizing antibody
NCI-CTCAE	National Cancer Institute – Common Terminology Criteria for Adverse Events
NIAID/FAAN	National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network
NIMP	non-investigational medical products
NPKUA	National PKU Alliance
NSAID	nonsteroidal anti-inflammatory drug
PAH	phenylalanine hydroxylase
PAL	phenylalanine ammonia lyase

Abbreviation	Definition
PDCO	European Medicines Agency's Paediatric Committee
PEG	polyethylene glycol
PFS	pre-filled syringe
Phe	phenylalanine
PIP	Paediatric Investigational Plan
PK	pharmacokinetics
PKU	phenylketonuria
PRN	pro re nata (as needed)
rAvPAL-PEG	PEGylated recombinant Anabaena variabilis phenylalanine ammonia lyase
RCT	randomized control trial
RDI	recommended daily intake
REMS	Risk Evaluation and Mitigation Strategy
SAE	serious adverse event
SAP	Statistical Analysis Plan
sBLA	supplemental Biologics License Application
SC	Study Completion
SD	standard deviation
SoA	Schedule of Activities
SOI	Statement of Investigator
TAb	total anti-drug antibody
Tyr	tyrosine
ULN	upper limit of normal
US	United States
WOCBP	Woman of Childbearing Potential

10.8 Appendix 8: Protocol Amendment History

The Protocol Amendment Summary of Changes Table for the current amendment is located directly before the Table of Contents (TOC).

Amendment 1 (global): 26 January 2022

Overall Rationale for the Amendment

The protocol is being amended in response to feedback received from the Food and Drug Administration (FDA) in a request for advice/information letter dated 2 December 2021, and in response to feedback received from the European Medicines Agency's Paediatric Committee (PDCO) in a Paediatric Investigational Plan (PIP) modification positive opinion. Additionally, minor typographic and consistency errors are also being corrected.

Section Number and Name	Description of Change	Brief Rationale
Synopsis Figure 1.2.2 (Dosing Schematic) 4.1 (Overall Design) 5.5 (Study Stopping Criteria) 6.5 (Dose Modification) Table 6.5.1 (Study Drug Dose Titration) 7.1 (Discontinuation of Study Treatment)	Clarified that pegvaliase should be discontinued in subjects who have not achieved an adequate response (< 600 µmol/L) after 16 weeks of continuous treatment at 60 mg/day	Response to FDA's advice/information request letter dated 02 December 02 2021, per US Prescribing Information.
Synopsis 5.1 (Inclusion Criteria)	Inclusion criterion wording amended to reflect that patients should have failed both sapropterin dihydrochloride and Phe-restricted diet, not one "and/or" the other.	Response to FDA's advice/information request letter dated 02 December 02 2021
Synopsis 5.1 (Inclusion Criteria) 5.3.1 (Meals and Dietary Restrictions) 8.1.2 (Total Dietary Protein Intake, Dietary Monitoring, and Dietary Protocol Restrictions)	Added "…Inclusion criterion to include the willingness for patients to maintain stable diet under supervision of study dietician or adequately trained designee per investigator during study participation"	As per agreement with PDCO, reflected in the PIP modification approval letter (EMEA-001951-PIP01-16-M02)
Synopsis Table 3.1 (Objectives and Endpoints)	Additional exploratory endpoint added with regards to the comparison of subjects' optimal maintenance pegvaliase dose across different age groups within the study	As per agreement with PDCO, reflected in the PIP modification approval letter (EMEA-001951-PIP01-16-M02)

Section Number and Name	Description of Change	Brief Rationale
Synopsis Table 1.3.1 (SoA Part 1, Pegvaliase Arm) Table 1.3.2 (SoA Part 2, Pegvaliase Arm) Table 1.3.4 (SoA Part 2, Diet-Only Arm) 4.1 (Overall Design) 4.2 (Scientific Rationale for Study Design) 4.3 (Justification of Dose and Dose Regimen) 6.5 (Dose Modifications)	Clarified dosing administration to occur in a hospital setting for the first 8 weeks and for all subsequent dose escalations.	As per agreement with PDCO, reflected in the PIP modification approval letter (EMEA-001951-PIP01-16-M02)
Synopsis 2.2 (Background) 4.1 (Overall Design) 4.2 (Scientific Rationale for Study Design) 5.1 (Inclusion Criteria)	Added the abbreviation “US” and the eligibility age criterion (12 to 15 years (EU), inclusive) in EU	Clarify the eligibility age criterion of the patient populations for EU and US given the pegvaliase approval in US for 18 years and older and in EU for 16 years and older
5.4 (Screen Failures)	Added clarifying language around subjects who are rescreened.	Makes it clear which rescreened subjects must performed all Screening Visits procedures and assessments when re-screened.
8.4 (Pharmacokinetics) Table 1.3.1 (SoA Part 1, Pegvaliase Arm) Table 1.3.2 (SoA Part 2, Pegvaliase Arm) Table 1.3.4 (SoA Part 2, Diet-Only Arm)	Added “A pre-dose sample should be collected within 2 hours of dosing”	PK window for pre-dose is now specified to ensure consistency in the timing of the PK samples for all subjects
Synopsis 9.4.2 (Primary Endpoints)	Stratification blood Phe measurement will include 2 blood Phe measurement performed during Screening/Run-In only	The Day 1 pre-dose blood Phe sample for stratification was included in error and has been removed. The two blood Phe samples performed during Screening/Run-In are adequate for treatment stratification.
Table 1.3.1 (SoA Part 1, Pegvaliase Arm) Table 1.3.3 (SoA Part 1, Diet-Only Arm)	Removed “and subject appraisal of metabolic control” from footnotes.	Text had been included in error
Table 1.3.3 (SoA Part 1, Diet-Only Arm)	Tryptase measurement added at Week 73	Was omitted in error

Section Number and Name	Description of Change	Brief Rationale
Table 1.3.1 (SoA Part 1, Pegvaliase Arm) Table 1.3.3 (SoA Part 1, Diet-Only Arm)	Column title changed from Study Day -28/1 to Study Day -28/-1	Correcting error of missing minus (–) sign
Table 1.3.1 (SoA Part 1, Pegvaliase Arm) Table 1.3.4 (SoA Part 2, Diet-Only Arm)	Urine pregnancy test added at HRV	For consistency with Table 1.3.2
Table 1.3.1 (SoA Part 1, Pegvaliase Arm)	Complement C3/C4 measurements added at Week 13 and Week 21	For alignment with Table 1.3.4 for Week 85 and Week 96

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CLINICAL STUDY PROTOCOL (EX-US [UNITED STATES])

Protocol Title:	A Phase 3 Multi-Center Study to Evaluate the Safety and Efficacy of Subcutaneous Injections of Pegvaliase in Adolescent Subjects (Ages 12-17) with Phenylketonuria Featuring an Open-Label Randomized Two-Arm (Active vs Diet-Only Control) Design
Protocol Number:	165-306
Amendment Number:	Amendment 2 (ex-US)
Compound:	pegvaliase (formerly BMN 165)
Short Title:	A Phase 3 Multi-Center Study to Evaluate the Safety and Efficacy of Subcutaneous Injections of Pegvaliase in Adolescent Subjects (Ages 12-17) with Phenylketonuria
Study Phase:	Phase 3
Sponsor Name:	BioMarin Pharmaceutical Inc.
Legal Registered Address:	105 Digital Drive Novato, CA 94949
Regulatory Agency Identifier Number(s):	IND: 076269 EudraCT: 2021-005058-27
Amendment 1 Date:	26 January 2022
Amendment 1 Date (ex-US):	06 June 2022
Amendment 2 Date:	23 October 2023
Amendment 2 Date (ex-US):	6 November 2023

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May not be divulged, published, or otherwise disclosed to others without prior written approval from BioMarin.
This study will be conducted according to the principles of Good Clinical Practice as described in the U.S. Code of Federal Regulations and the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use guidelines, including the archiving of essential documents.

I have read this study protocol and agree to conduct this study in compliance with the protocol, all applicable regulations, guidelines, and other information supplied to me.

Investigator Signature Date

Printed name: _____

Accepted for the Sponsor:

PI

PI Medical Monitor Signature

PI

Date

Printed name: PI RN, MS PI Clinical Science

Medical monitor contact information is available in Appendix 3, Section 10.3.

PROTOCOL AMENDMENT SUMMARY OF CHANGES TABLE

DOCUMENT HISTORY	
Document	**Approval date**
Amendment 2 (ex-US)	6 November 2023
Amendment 2	23 October 2023
Amendment 1 (ex-US)	06 June 2022
Amendment 1	26 January 2022
Original Protocol	22 September 2021

Amendment 2 (ex-US) (6 November 2023):

Overall Rationale for the Amendment

The protocol is being amended to include clinical outcome assessments (COAs) and to capture various administrative changes to further clarify some aspects of the protocol noted since the implementation of Amendment 1. Additionally, minor typographic and consistency errors are also being corrected.

Section Number and Name	Description of Change	Brief Rationale
1.1 Synopsis 1.3 Schedules of Activities Table 3.1 Objectives and Endpoints 8.1.3.1 Neurocognitive Assessments 8.1.3.2 Global Impression Scales 11 References	Added clinical outcome assessment (COA) exploratory endpoints: PKU-SSIS v2.0 (adolescent version) and accompanying global items (Patient Global Impression of Severity/Change [PGIS/C], Caregiver Global Impression of Severity/Change [CaGIS/C], and Clinician Global impression of Severity [CGIS].	The PKU-SSIS v2.0 (adolescent version) is a newly developed PKU-specific patient-reported outcome (PRO) measure to assess symptoms and impacts of PKU in adolescents with PKU. Global impression items are included to support validation of the PKU-SSIS in this patient population.
Table 2.3.1.1 Risks Associated with Study Drug 6.5 Dose Modification	Clarified that a pegvaliase dose reduction is required in the event of hypophenylalaninemia.	Previous language proposed that either dose reduction or dietary protein changes were acceptable risk mitigation for normalization of hypophenylalaninemia. Dose reduction must be performed, and a dietary protein change is an additional option for managing hypophenylalaninemia
1.3 Schedules of Activities 8.4 Pharmacokinetics	Added PK collection sample time windows.	To allow flexibility for specimen collection times.

Section Number and Name	Description of Change	Brief Rationale
1.1 Synopsis Figure 1.2.2 Dosing Schematic 1.3 Schedules of Activities 4.1 Overall Design 6.5 Dose Modification 8 Study Assessments and Procedures	Duration of Part 2 of the study extended from 80 weeks to 142 weeks (total study duration 214 weeks)	Duration of Part 2 extended due to longer enrollment period
1.1 Synopsis Table 6.1.1 Study Treatment 6.5 Dose Modification Table 6.5.1 Study Drug Dose Titration Schedule	Include additional option of 5 mg dose level during induction/titration	Provide investigators with discretion to use an additional dosing option during induction/titration
1.3 Schedules of Activities Table 10.2.1 Clinical Laboratory Tests	Urine N-methylhistamine test added to Day 1 and hypersensitivity visit in the Schedules of Activities	Added to clarify discrepancy within protocol
Figure 1.2.2 Dosing Schematic 6.5 Dose Modification Table 6.5.1 Study Drug Dose Titration Schedule	Previous requirement for blood Phe to be ≤ 360 µmol/L for dose escalations has been changed from a requirement to a recommendation	To be consistent with investigator discretion
1.1 Synopsis 1.3 Schedules of Activities Table 3.1 Objectives and Endpoints 8.1.2 Total Dietary Protein Intake, Dietary Monitoring, and Dietary Protocol Restrictions 9.4.3 Secondary Endpoints	Total dietary protein should be measured in grams per kilogram (g/kg)	Clarified total dietary protein unit

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1 PROTOCOL SUMMARY

1.1 Synopsis

Protocol Title: A Phase 3 Multi-Center Study to Evaluate the Safety and Efficacy of Subcutaneous Injections of Pegvaliase in Adolescent Subjects (Ages 12-17) with Phenylketonuria Featuring an Open-Label Randomized Two-Arm (Active vs Diet-Only Control) Design

Short Title: A Phase 3 Multi-Center Study to Evaluate the Safety and Efficacy of Subcutaneous Injections of Pegvaliase in Adolescent Subjects (Ages 12-17) with Phenylketonuria

Study Design Rationale:

This Phase 3 multicenter study is designed to evaluate the safety and efficacy of pegvaliase administered daily to adolescent participants (ages 12 to 17 years old (US), inclusive, and 12 to 15 years old (ex-US [United States]), inclusive) with phenylketonuria (PKU). Participants will be randomized in a 2:1 ratio to the active (pegvaliase) and control (diet-only) treatment arms, respectively, with 36 participants receiving pegvaliase and 18 participants managing their PKU with diet alone. The primary efficacy endpoint is the change from baseline in blood phenylalanine (Phe) following 72 weeks on study (the end of Part 1). Beginning Week 73, participants in the active (pegvaliase) treatment arm will continue to receive pegvaliase in the Extension Phase (Part 2) for up to an additional 142 weeks (through Week 215). Participants in the control (diet only) treatment arm will initiate pegvaliase treatment beginning Week 73 and, from Weeks 73 through 215, follow the same dosing and assessment schedule that the active participants followed.

A randomized control trial (RCT) design, with diet-only management of PKU as the control, will allow for robust data collection and minimize the risk of confounding factors influencing the results. An open-label diet-only control arm is the best comparator for this age group as daily placebo injections would place undue burden on adolescent PKU patients.

In Part 1 (through the Week 73 visit), randomized participants in both the control (diet-only) and active (pegvaliase) treatment arms will be required to maintain the same level of protein intake they established during Screening/Run-in. In Part 2, participants in the control (diet-only) arm must continue to maintain this prescribed level of protein intake, whereas participants randomized to the active (pegvaliase) arm whose blood Phe concentration is ≤ 360 µmol/L for a minimum of 4 weeks may adjust dietary protein intake based on individual participant response to pegvaliase and guidance from the investigator. Participants

are required to maintain a consistent level of protein intake during the study to ensure that the safety and efficacy endpoints are attributable to pegvaliase rather than to changes in dietary protein intake.

Pegvaliase will be subcutaneously injected in a hospital setting for the first 8 weeks and thereafter at the clinic or at home by a trained participant, caregiver, adult observer, or visiting healthcare provider. Pegvaliase will also be injected in a hospital setting for any subsequent dose escalations. Administration at home makes daily treatment less burdensome for participants, thereby improving treatment compliance.

An adult observer is required for a minimum of 1 hour post injection throughout the study. An adult observer is defined as someone who will be present with the participant during and after pegvaliase administration, is able to recognize the signs and symptoms of an anaphylaxis episode, and is able to call for emergency medical support and administer epinephrine, if warranted. The requirement for an adult observer is included to ensure that participants self-administering pegvaliase would have support in recognizing and quickly responding to signs and symptoms of anaphylaxis.

The primary efficacy endpoint is the change from treatment naïve baseline in blood Phe following 72 weeks on study (the end of Part 1). Blood Phe level is the established clinical marker used to diagnose PKU and assess disease control, and reduction of blood Phe concentration to target levels is the universally acknowledged treatment goal for PKU patients.

Objectives and Endpoints:

The primary, secondary, and exploratory objectives and associated endpoints are shown below. Refer to the Schedule of Activities (SoA) for the complete listing of assessment timepoints.

Objectives	Endpoints
Primary	
To evaluate the efficacy of pegvaliase in adolescent participants with PKU	The primary efficacy endpoint is change in blood Phe concentration from treatment naïve baseline following 72 weeks on study. Change in blood Phe from baseline is compared between participants in the active (pegvaliase) and the control (diet-only) treatment arms.
To evaluate the safety of pegvaliase in adolescent participants with PKU	• Adverse events (AEs), including serious AEs (SAEs) • Clinical laboratory tests (chemistry, hematology, and urinalysis) results • Vital signs • Physical examination (including height, weight) • Electrocardiogram (ECG) test results • Immunogenicity test results (anti-PEG IgG, anti-PEG IgM, anti-PAL IgG, anti-PAL IgM, Tab, Nab, and anti-pegvaliase IgE [hypersensitivity reaction visits only]).
Secondary	
To characterize total dietary protein intake in adolescent participants with PKU after pegvaliase treatment	Total dietary protein intake must remain stable for change in blood Phe, the primary endpoint, to be accurately assessed. To gauge the stability of total protein intake, the change from baseline at Week 73 in total dietary protein intake (measured in grams/kilogram [g/kg]) (defined as the sum of the protein intake from medical food and intact food) will be measured.
To characterize the pharmacokinetics (PK) of pegvaliase in adolescent participants with PKU	Trough plasma PK samples will be collected and analyzed as specified in the SoA. PK parameters assessed with intensive PK sampling will include $AUC_{0\text{-}24hr,ss}$, $C_{max,ss}$, $T_{max,ss}$, $t_{1/2}$, $CL_{ss/F}$, and $V_{dz/F}$. For participants randomized to the active (pegvaliase) treatment arm, intensive PK sampling will be performed at Week 73 and for dose escalations to 20, 40, and 60 mg/day. For participants randomized to the control (diet-only) treatment arm, there are no PK assessments in Part 1. In Part 2, intensive PK sampling will be performed at Week 145 and for dose escalations to 20, 40, and 60 mg/day.

Objectives	Endpoints
Exploratory Objectives	
To evaluate the effect of pegvaliase treatment on neurocognitive outcomes in adolescent participants with PKU.	• The ADHD Rating Scale IV inattention subscale (ADHD RS-IV) rated by the parent/guardian and performed at Baseline (Day 1, pre-dose) and every 12 weeks throughout the study. • The Behavior Rating Inventory of Executive Function, Second Edition (BRIEF2) rated by the parent/guardian and performed at Baseline (Day 1, pre-dose) and every 12 weeks throughout the study. • Change from baseline (or first administration) in Phenylketonuria Symptom Severity and Impacts Scale (PKU-SSIS) Version 2.0 scores.
To assess changes in PKU symptoms and impacts	• Change from baseline (or first administration) in global severity items (Patient Global Impression of Severity [PGIS], Caregiver Global Impression of Severity [CaGIS], and Clinician Global Impression of Severity [CGIS]). • Change in global change items (Patient Global Impression of Change [PGIC] and Caregiver Global Impression of Change [CaGIC]).
To compare participants' optimal maintenance pegvaliase dose across different age groups within the study	• Time to participants' optimal maintenance dose compared to older age subsets • Number of participants who require the highest (60 mg/day) maintenance dose • Percentage/number of participants in the blood Phe target ranges of < 600 µmol/L and < 360 µmol/L.
Explore the biochemical, molecular, and cellular aspects of PKU	Blood will be collected to evaluate biochemical, molecular, and cellular aspects of PKU and to develop the assays used for these evaluations.
PAH genotyping	Testing will be performed on samples collected Day 1 pre-dose where allowed by local health authorities and the EC/IRB.

Overall Design:

Study 165-306 is an open-label, two-arm, randomized control evaluation of pegvaliase in approximately 54 participants (ages 12 to 17 years old (US), inclusive, and 12 to 15 years old (ex-US), inclusive) with diet only as the control arm. To be eligible for the study, potential participants must meet all eligibility requirements described in the protocol, including the following selected key inclusion criteria:

- Is 12 to 17 years old (US), inclusive, or 12 to 15 years old (ex-US), inclusive, at the start of the Screening/Run-in Period (Day 28).

- Diagnosis of PKU and failure to maintain recommended blood Phe levels on existing management (defined as having failed sapropterin dihydrochloride treatment and Phe-restricted diet) demonstrated by 2 blood Phe concentration measurements > 600 µmol/L during the Screening/Run-in Period (7 to 10 days between blood Phe assessments) and average blood Phe concentration > 600 µmol/L over the past 12 months (per available data).
- Willing and able to maintain and adjust dietary and medical protein food intake according to the study protocol and under the supervision of study dietician or adequately trained designee per investigator during study participation.
- An adult (≥ 18 years of age) has been identified who is willing and competent to observe the participant during study drug administration and for a minimum of 1 hour following administration. A home healthcare nurse may perform the study drug observations consistent with local regulatory and site requirements.

Selected key exclusion criteria include the following:

- Have a history of a severe systemic hypersensitivity reaction or recurrence of a mild to moderate acute systemic hypersensitivity reaction to any of the excipients or another PEGylated medicinal product.
- Potential participants are not eligible if they are unable to identify and/or communicate to others that they are experiencing symptoms of potential anaphylaxis due to cognitive impairment or other reasons.

Following enrollment, participants will be randomized 2:1 to the active (pegvaliase) and control (diet-only) treatment arms, with 36 participants receiving daily pegvaliase and 18 participants managing PKU by diet alone. Treatment assignment will be stratified by Screening/Run-In blood Phe (mean of the 2 pre-treatment blood Phe assessments performed during the Screening/Run-in period) of ≤ 1000 µmol/L or > 1000 µmol/L. The primary efficacy endpoint is the change from baseline blood Phe following 72 weeks on study (the end of Part 1). Participants in the active and control arms will follow the same visit schedules and perform the same assessments throughout the 72-week Primary Treatment Phase (Part 1) except that the control participants will not be receiving pegvaliase so will not have PK draws or immunogenicity assessments.

After providing informed consent, participants will enter the Screening/Run-in period, which will last 4 weeks and include 2 assessments of blood Phe levels, 7 to 10 days apart, to determine eligibility. Following confirmation that the 2 blood Phe results are > 600 µmol/L and all other eligibility criteria have been met, participants may be enrolled in the study on Day 1. During the Screening/Run-in and the Primary Treatment Phase (Part 1), participants will be required to maintain stable protein intake from both medical food and/or intact food.

The duration of Part 1 is 72 weeks, during which pegvaliase treatment will be initiated for participants in the active arm using an Induction/Titration/Maintenance (I/T/M) dosing regimen. The I/T/M dosing regimen consists of Induction/Titration Dosing (9 weeks) and Maintenance Dosing. Required hospital setting visits during Part 1 will occur for all dosing visits for the first 8 weeks, every 4 weeks thereafter, and when the pegvaliase dose is escalated to 20, 40, or 60 mg/day. The assessments performed at the clinic visits include measurement of blood Phe, neurocognitive assessments, global impression items, PK blood draws (active participants only), and safety assessments.

Beginning Week 73, participants in the active treatment arm will continue to receive pegvaliase in the Extension Phase (Part 2) for up to 142 additional weeks (through Week 215). Beginning Week 73, participants in the control treatment arm will initiate pegvaliase treatment and, from Week 73 through Week 215, will follow the same schedule for dosing, study visits, and assessments that the active arm participants followed.

Brief Summary:

The purpose of Study 165-306 is to evaluate the safety and efficacy of pegvaliase and characterize the PK of pegvaliase in adolescent participants with PKU. Study details include:

Condition/Disease: PKU is a rare autosomal recessive genetic disorder caused by mutations in the phenylalanine hydroxylase (PAH) gene, leading to an absence or deficiency in PAH enzyme activity and subsequent elevation of the amino acid phenylalanine (Phe) in the blood, known as hyperphenylalaninemia (HPA) (Vockley 2014; Mitchell 2011). Deficiency of PAH results in an abnormally elevated concentration of Phe, which is toxic to the brain. High Phe levels during infancy and early childhood cause profound neurocognitive and developmental defects, and poorly controlled blood Phe levels in older children and adults are associated with learning disabilities, attention deficit hyperactivity disorder, behavioral problems, and psychiatric symptoms.

Study Hypothesis: The study hypothesis is that participants receiving pegvaliase will show a greater reduction in blood Phe following 72 weeks on study compared to participants in the diet-only control group.

Study Duration: The study duration is up to 222 weeks for participants randomized to active (pegvaliase) treatment. This includes a 4-week Screening/Run-in period, up to 214 weeks of pegvaliase treatment in Parts 1 and 2, and the Study Completion/Early Termination (SC/ET) visit 4 weeks after the final dose of pegvaliase. The study duration is also 222 weeks for participants randomized to control (diet-only) treatment. This includes a 4-week Screening/Run-in period, 72 weeks managing PKU with diet alone while following the Part 1

assessment schedule, 142 weeks of pegvaliase treatment following the same assessment schedule that participants in the active treatment arm followed, and the SC/ET visit 4 weeks after the final dose of pegvaliase.

Treatment Duration: The pegvaliase treatment duration will be 214 weeks for participants randomized to the active (pegvaliase) treatment arm (n=36). The pegvaliase treatment duration will be 142 weeks for participants randomized to the control (diet-only) treatment arm (n=18).

Health Measurement/Observation: Reduction in blood Phe.

Visit Frequency: For both active and control participants, hospital setting visits in Part 1 will occur for all dosing visits for the first 8 weeks, then every 4 weeks through Week 73 and for all dose escalations. For participants randomized to active (pegvaliase) treatment, clinic visits in Part 2 will occur every 4 weeks through Week 215 and for all dose escalations. For participants randomized to control (diet-only) treatment, hospital setting visits in Part 2 will occur for all dosing visits for the first 8 weeks, then every 4 weeks through Week 215. For participants randomized to active (pegvaliase) treatment in Part 1 and participants randomized to control (diet-only) treatment in Part 2, hospital setting visits will also occur when their dose level is escalated to 20, 40, and 60 mg/day.

Number of Participants:

Approximately 54 participants will be enrolled and randomized to the pegvaliase or diet-only groups in a 2:1 ratio. Assuming the mean (SD) reduction in Phe following 72 weeks on study is 640 (570) µmol/L in the pegvaliase group and 100 (250) µmol/L in the diet-only group, a total sample size of 54 participants will provide more than 90% power to detect a treatment difference, based on the two-sample T-test with unequal variance and a significance level of 0.05 (two sided). The assumption of treatment effect is based on clinical data from previous pegvaliase studies and a PKU registry study. See the Statistical Analysis Plan (SAP) for additional details on the sample size calculation.

Approximately 72 participants will be screened for study participation such that approximately 54 participants will be enrolled in the study.

Note: Potential participants who are screened for the purpose of determining eligibility for the study, but do not participate in the study, are not considered enrolled.

Treatment Groups and Duration:

The duration of the Primary Treatment Phase (Part 1) is 72 weeks, during which pegvaliase treatment will be initiated for participants randomized to the active (pegvaliase) treatment

arm using an I/T/M dosing regimen. Induction will consist of a 4-week period when participants randomized to active drug will receive pegvaliase subcutaneously with a pre-filled syringe (PFS) at a fixed dose of 2.5 mg/week and during which the dosing regimen will not be modified in response to blood Phe concentration. Once participants complete induction they will titrate up to a dose of 10 mg/day (investigators may, at their discretion, include dosing at a 5 mg/day dose level). The dosing frequency will be increased gradually during titration dosing to daily (7 days/week). During Maintenance Dosing the dose will be increased to 20 mg/day, 40 mg/day, and 60 mg/day depending upon individual blood Phe lowering efficacy (per the investigator's discretion). The dose should be increased to 40 mg/day if blood Phe is > 360 µmol/L (recommended) after 24 weeks dosing at 20 mg/day and increased to 60 mg/day if blood Phe is > 360 µmol/L (recommended) after 16 weeks dosing at 40 mg/day. If a participant's blood Phe is > 600 µmol/L after 16 weeks of continuous treatment with pegvaliase at 60 mg/day, pegvaliase should be permanently discontinued. BioMarin recommends that the target blood Phe levels should be based on individual needs per investigator's discretion, with a target blood Phe ≤ 600 µmol/L or lower. During the Primary Treatment Phase (Part 1), participants randomized to the control (diet-only) treatment arm will have their PKU managed by diet alone.

Following completion of Part 1, participants randomized to active (pegvaliase) treatment will enter the Extension Phase (Part 2), during which participants will continue dosing with pegvaliase at their optimal maintenance dose level for up to an additional 142 weeks (through Week 215) in order to evaluate the long-term safety and efficacy of pegvaliase in adolescent participants with PKU. Following completion of Part 1 (Week 72), participants randomized to control (diet-only) treatment will initiate pegvaliase treatment and, from Week 73 through Week 215, will follow the same schedule for dosing, study visits, and assessments that the active arm participants followed.

Data Monitoring Committee:

An independent Data Monitoring Committee (DMC) will monitor the safety of study participants. The DMC is an independent committee that acts in an advisory capacity to BioMarin.

1.2 Study Schema

Figure 1.2.1: Active (Pegvaliase) vs Control (Diet-Only) Treatment Arms (Randomized Treatment Assignment)

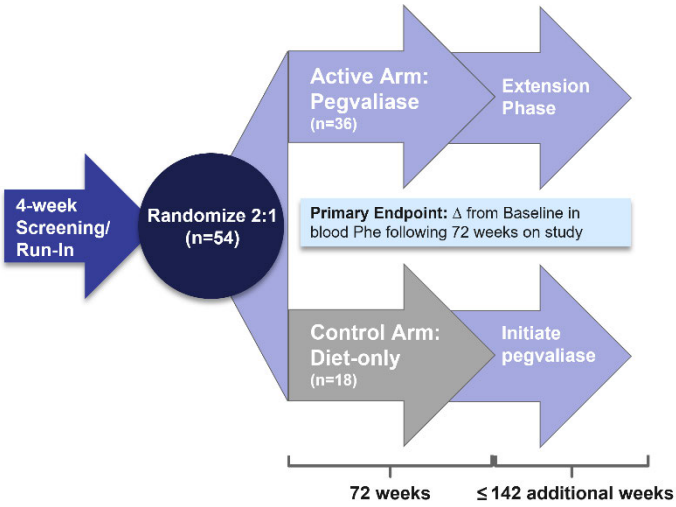
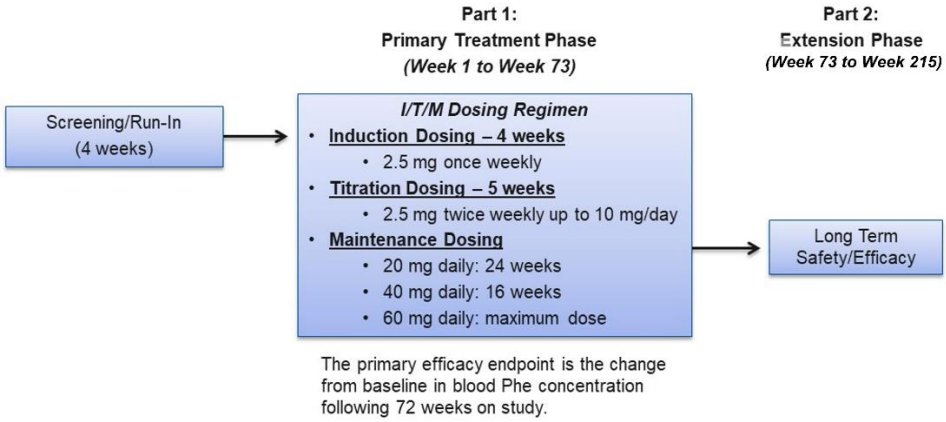


Figure 1.2.2: Dosing Schematic for Active (Pegvaliase) Treatment Arm



Note: The 18 control (diet only) participants will follow the Part 1 assessment schedule (except for pegvaliase dosing and PK/immunogenicity assessments) from Weeks 1 through Week 73 and then will initiate pegvaliase treatment and, from Week 73 through Week 215, will follow the same schedule for dosing, study visits, and assessments as the active arm participants.

Prior to dose escalation, the minimum amount of time pegvaliase can be dosed at 20 mg/day before escalating is 24 weeks and the minimum amount of time at 40 mg/day before escalating is 16 weeks. The dose should be increased to 40 mg/day if blood Phe is > 360 µmol/L (recommended) after 24 weeks dosing at 20 mg/day, and increased to 60 mg/day if blood Phe is > 360 µmol/L (recommended) after 16 weeks dosing at 40 mg/day. If a participant's blood Phe is > 600 µmol/L after 16 weeks of continuous treatment with pegvaliase at 60 mg/day, pegvaliase should be permanently discontinued.

1.3 Schedule of Activities (SoA)

Table 1.3.1: Schedule of Activities for Part 1 (Participants Randomized to Pegvaliase Treatment Arm)

Assessment [a]	Study Week	Screen[b]	1	2	3	4	5	6	7	8	9	11	13	15	17	19	21	25	29	33	37	41	45	49	53	57	61	65	69	73	HRV [c]	SC/ET [d]
	Study Day	-28/-1	1	8	15	22	29	36	43	50	57	71	85	99	113	127	141	169	197	225	253	281	309	337	365	393	421	449	477	505		
	Window (Days)			±3	±3	±3	±3	±3	±3	±3	±3	±3	±7	±3	±7	±3	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7		
Informed consent		X																														
Drug administration training [e]			X																													
Study drug observer training [f]		X																														
Demographics		X																														
Medical history [g]		X																														
HIV, hepatitis B and C screens [h]		X																														
PAH genotyping [i]			X																													
12 lead ECG		X	X																													X
Physical examination [j]		X	X	X	X	X	X	X	X	X	X		X		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Weight		X	X	X	X	X	X	X	X	X	X		X		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Height		X	X				X				X		X		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
Vital signs		X	X	X	X	X	X	X	X	X	X		X		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Clinical safety laboratory tests [k]		X	X	X	X	X	X	X	X	X	X		X		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
C-reactive protein		X	X								X				X			X		X		X		X		X		X		X	X	X
Erythrocyte sedimentation rate [l]		X	X																													X
Serum cortisol [m]			X															X						X						X		X
Complements C3 and C4			X				X				X		X		X		X	X		X		X		X		X		X		X	X	X
Tryptase			X																												X	
Urine albumin/creatinine ratio [n]			X								X				X			X		X		X		X		X		X		X	X	X
Urine N-methylhistamine			X																												X	

	Study Week	Screen[b]	1	2	3	4	5	6	7	8	9	11	13	15	17	19	21	25	29	33	37	41	45	49	53	57	61	65	69	73	HRV[c]	SC/ET[d]
	Study Day	-28/-1	1	8	15	22	29	36	43	50	57	71	85	99	113	127	141	169	197	225	253	281	309	337	365	393	421	449	477	505		
Assessment [a]	Window (Days)			±3	±3	±3	±3	±3	±3	±3	±3	±3	±7	±3	±7	±3	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7		
Urine pregnancy test [o]		X	X	X	X	X	X	X	X	X	X		X		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
3-day diet diary [p]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
PK (plasma pegvaliase) [q]			X				X				X		X		X		X	X		X		X		X		X		X			X
Blood for exploratory analyses [i]			X				X				X		X		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
Blood Phe, tyrosine [r]		X [b]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Immunogenicity assessments [s]			X	X	X	X	X	X	X	X	X		X		X		X	X		X		X		X		X		X		X	X
ADHD RS-IV [t]			X										X					X			X			X			X			X		X
BRIEF2 [t]			X										X					X			X			X			X			X		X
PKU-SSIS [u,z]			X										X					X			X			X			X			X		X
PGIS [u,z]			X										X					X			X			X			X			X		X
PGIC [u]																					X									X		X
CaGIS [t,z]			X										X					X			X			X			X			X		X
CaGIC [t]																					X									X		X
CGIS [v,z]			X										X					X			X			X			X			X		X
Weekly Phone Call (non-visit weeks) [w]																																
Adverse events [x]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant medication [x]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Administer study drug [y]			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		

ADHD RS-IV, attention deficit hyperactivity disorder rating scale IV; BRIEF2, Behavior Rating Inventory of Executive Function, Second Edition; EC/IRB, Ethics Committee/Institutional Review Board; ECG, electrocardiogram; eCRF, electronic Case Report Form; HRV, hypersensitivity reaction visit; PAH, phenylalanine hydroxylase; PE, physical examination; PK, pharmacokinetics; PKU, phenylketonuria; SC/ET, Study Completion/Early Termination visit.

[a] Scheduled visits take place at the hospital setting (for all dosing visits for the first 8 weeks, and for all dose escalation visits), clinic, or, with the approval of the investigator or designee, virtually. For virtual visits the investigator/designee performs assessments while online with the participant, apart from specimen collections and vital signs, which are performed by home healthcare personnel. Non-clinic visits are via telephone. Assessments are performed pre-dose unless otherwise specified.

[b] After written informed consent, screening assessments should be performed within 28 days prior to Part 1, Day 1. Participants will be assessed for blood Phe concentration during Screening/Run-in with 2 measurements 7 to 10 days apart. These 2 blood Phe measurements should be scheduled to occur: (1) in the Day -25 to Day -23 window; and (2) 7 to 10 days later (ie, in the Day -18 to Day -13 window).

[c] If the investigator determines that the NCI-CTCAE Grade ≥ 3 hypersensitivity reaction is related to administration with study drug, the participant will be asked to return to the clinic within 24 hours of event onset for a hypersensitivity reaction visit (HRV). Sampling for anti-pegvaliase IgE should be performed 8 to 24 hours after event onset and before the next dose of study drug.

[d] Participants should perform the Study Completion (SC) Visit 4 weeks after the last dose of study drug or after completion of study participation, whichever occurs last. For participants who terminate from the study early, the Early Termination (ET) Visit occurs within 4 weeks of the last study drug dose or after withdrawal from study participation, whichever occurs last. Participants who discontinue from study drug early will be asked to continue to complete the remaining study assessments until study completion as long as such continued participation will not detrimentally affect the health, safety, and welfare of the participant per investigator determination.

[e] Once participant/caregiver competence is documented, the participant/caregiver administers study drug daily.

[f] Must be completed prior to administration of the first dose of study drug. Additional observers identified after screening must be trained.

[g] PKU history includes highest blood Phe (and age).

[h] Participants with documented negative results within the last 4 weeks do not need to be retested.

[i] Sample collection should be performed where allowed by country-specific regulations and EC/IRB.

[j] A full physical examination (PE) will be performed at Screening, Baseline, Week 73, and the SC/ET visit. A brief PE will be performed at other scheduled visits. A full PE will include, at a minimum, general appearance (head, eyes, ears, nose, and throat) and an assessment of cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, musculoskeletal, and neurologic systems. A brief PE may be performed at the discretion of the investigator based on the participant's clinical condition and may include, but is not limited to, general appearance and an assessment of cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, musculoskeletal, and neurologic systems.

[k] It is recommended that urine samples are obtained as a first or second morning void. In the event of elevated urinary protein on a test result, a repeat urinalysis should be performed. This repeat urine sample must be performed in the morning at the first or second morning void to allow for accurate test results and may be performed by a home healthcare nurse.

[l] To be done by a local laboratory.

[m] Serum cortisol samples should be taken before study drug administration in the morning before 10:00 am. A home healthcare nurse may collect samples for participant convenience. If 2 results are low and abnormal, the participant will be asked to perform a low-dose conventional ACTH stimulation test or the ACTH test method based on the clinical practice at the site.

[n] It is recommended that urine samples be obtained as a first or second morning void. Participants with a confirmed urine/albumin creatinine ratio of ≥ 100 mg/g should be referred to a nephrologist for consultation if results were within normal range at Baseline. Participants who had elevated results at Baseline followed by a confirmed subsequent increase of 100-200 mg/g from Baseline should also be referred to a nephrologist for consultation.

[o] To be done locally (if applicable). If urine pregnancy test is positive or equivocal, serum pregnancy test (central laboratory) must be done.

[p] Participants should record at home all food, beverages, special low-protein foods, and medical foods consumed during the 3 consecutive days preceding a study visit. No change in protein (dietary and medical food) intake is allowed in Part 1. During Screening/Run-in, 3-day diet diary assessments are performed 3 times, each time covering the 3-day periods prior to blood Phe assessments (and collected on the day of those assessments). The 3 Screening/Run-in blood Phe assessments are scheduled to occur (1) at Day -25 to Day -23; (2) 7 to 10 days later (ie, in the Day -18 to Day -13 window); and (3) at Day 1 pre-dose. Thus, the 3 day diet assessments will assess diet for (1) the 3 days within the Day -28 to Day -23 window immediately prior to the first blood Phe assessment; (2) the 3 days within the Day -21 to Day -15 window immediately prior to the second blood Phe assessment; and (3) for Day -3 to Day 1.

[q] Blood is collected before study drug administration (pre-dose) within 2 hours of dosing. Intensive PK sampling will be performed at Week 73 and at dose escalation visits in all participants. Samples will be taken at pre-dose (within 2 hours of dosing); at 2, 4, 8, and 12 hours (± 30 minutes) post-dose; and 24 hours (± 2 hours) post dose. The 24-hour sample will be taken prior to the next daily dose.

[r] Blood is collected for blood Phe analysis after fasting a minimum of 2.5 hours. In addition to the blood draws for blood Phe analysis collected in the clinic and shown in the SoA, there are 5 required blood collections that may be drawn either at the clinic or elsewhere (eg, at home). These are to be collected at Weeks 11, 15, and 19, and 2 weeks prior to the planned escalations to 40 and 60 mg/day. Three (3) Day diet diary data collection must be done prior to all Phe collections.

[s] Immunogenicity assays include total anti-pegvaliase antibodies (TAb), anti-PAL IgG, anti-PAL IgM, anti-PEG IgM, anti-PEG IgG, and neutralizing antibodies (NAb). At the HRV, only anti-pegvaliase IgE will be assessed.

[t] Completed by parent/guardian. Assessments should be completed before other study visit assessments (eg, vital signs, blood draw for blood Phe, and study drug administration).

[u] Completed by participant. Assessments should be completed before other study visit assessments (eg, vital signs, blood draw for blood Phe, and study drug administration).

[v] Completed by the investigator or designee clinician. Assessments should be completed before other study visit assessments (eg, vital signs, blood draw for blood Phe, and study drug administration). Visits where CGIS will be assessed should take place in the hospital setting or clinic.

[w] Between scheduled visits, the clinic staff will contact the participant via phone weekly to monitor if the participant is experiencing problems with drug administration, to ask about any AEs or concomitant medications, and to answer questions.

[x] AEs and concomitant medications should be noted whenever a participant is assessed by study personnel. Following signed informed consent and prior to the first dose of pegvaliase, only SAEs associated with study procedures are collected. After the first dose all AEs and SAEs are collected until 4 weeks after the last study drug dose

or the Early Termination Visit, whichever occurs last. If there is a severe (Grade 3) injection site reaction, the participant may be referred to a dermatologist for consultation and a skin biopsy (optional; if clinically indicated). If photographs of the severe injection site reaction are taken, they may be collected by the sponsor.

[y] Participants must be premedicated with an H1 antagonist, and an H2 antagonist, and an antipyretic approximately 2 to 3 hours prior to each dose of study drug until they reach the Maintenance Phase, defined as when participants achieve blood Phe ≤ 600 µmol/L while they are on a stable dose of pegvaliase. Premedication may be considered during the Maintenance Phase at the investigator's discretion, eg, approximately 2 to 3 hours prior to study drug for 1 week upon reintroduction of study drug, upon resolution of an AE, following any dose interruption of ≥ 4 days, and for any dose increases in the Extension Phase. Participants may also be premedicated at any other time in the study per investigator discretion. When an NSAID is administered as a premedication, it should be given with food. For non-clinic visits, participants are asked about study drug administration.

[z] Initial assessments of the PKU-SSIS, PGIS, CaGIS, and CGIS will be at the first visit following implementation/adoption of Protocol Amendment 2.

Table 1.3.2: Schedule of Activities for Part 2 (Extension Phase) Weeks 73-149 (Participants Randomized to Pegvaliase Treatment Arm)

Assessment [a]	Study Week	73[b]	77	81	85	89	93	97	101	105	109	113	117	121	125	129	133	137	141	145	149	HRV[c]	SC/ET[d]
	Study Day	505	533	561	589	617	645	673	701	729	757	785	813	841	869	897	925	953	981	1009	1037		
	Window (Days)	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7		
Study drug observer training [e]																							
12 lead ECG																							X
Physical examination [f]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Weight	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Height	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			X
Vital signs	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Clinical safety laboratory tests [g]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
C-reactive protein	X		X		X		X		X		X		X		X		X		X		X	X	X
Erythrocyte sedimentation rate [h]																							X
Serum cortisol [i]	X						X						X										X
Complements C3 and C4	X		X		X		X		X		X		X		X		X		X		X	X	X
Tryptase																						X	
Urine albumin/creatinine ratio [j]	X		X		X		X		X		X		X		X		X		X		X	X	X
Urine N-methylhistamine																						X	
Urine pregnancy test [k]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
3-day diet diary [l]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			X
PK (plasma pegvaliase) [m]	X		X		X		X		X		X		X		X		X		X				X
Blood for exploratory analyses [n]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			X
Blood Phe, tyrosine [o]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Immunogenicity assessments [p]	X		X		X		X		X		X		X		X		X		X		X	X	X
ADHD RS-IV [q]	X			X			X			X			X			X			X				X
BRIEF2 [q]	X			X			X			X			X			X			X				X
PKU-SSIS [r]	X			X			X			X			X			X			X				X

Assessment [a]	Study Week	73[b]	77	81	85	89	93	97	101	105	109	113	117	121	125	129	133	137	141	145	149	HRV[c]	SC/ET[d]
	Study Day	505	533	561	589	617	645	673	701	729	757	785	813	841	869	897	925	953	981	1009	1037		
	Window (Days)	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7		
PGIS [r]		X			X			X			X			X			X			X			X
PGIC [r]		X									X									X			X
CaGIS [q]		X			X			X			X			X			X			X			X
CaGIC [q]		X									X									X			X
CGIS [s]		X			X			X			X			X			X			X			X
Weekly Phone Call (non-visit weeks) [t]																							
Adverse events [u]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant medication [u]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Administer study drug [v]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		

ADHD RS-IV, attention deficit hyperactivity disorder rating scale IV; BRIEF2, Behavior Rating Inventory of Executive Function, Second Edition; ECG, electrocardiogram; eCRF, electronic Case Report Form; HRV, hypersensitivity reaction visit; PE, physical examination; PK, pharmacokinetics; PKU, phenylketonuria; SC/ET, Study Completion/Early Termination visit.

[a] Scheduled visits take place at the hospital setting (for all dose escalation visits and visits where the CGIS assessments will be performed), clinic, or, with the approval of the investigator or designee, virtually. For virtual visits the investigator/designee performs assessments while online with the participant, apart from specimen collections and vital signs, which are performed by home healthcare personnel. Non-clinic visits are via telephone. Assessments are performed pre-dose unless otherwise specified.

[b] The Week 73 visit marks both the end of the Primary Treatment Phase (Part 1) and the beginning of the Extension Phase (Part 2). The pre-dose assessments constituting the end of the Primary Treatment Phase and the administration of study drug constituting the beginning of the Extension Phase are shown in the Week 73 columns of both Table 1.3.1 and Table 1.3.2.

[c] If the investigator determines that the NCI-CTCAE Grade ≥ 3 hypersensitivity reaction is related to administration with study drug, the participant will be asked to return to the clinic within 24 hours of event onset for a hypersensitivity reaction visit (HRV). Sampling for anti-pegvaliase IgE should be performed 8 to 24 hours after event onset and before the next dose of study drug.

[d] Participants should perform the Study Completion (SC) Visit 4 weeks after the last dose of study drug or after completion of study participation, whichever occurs last. For participants who terminate from the study early, the Early Termination (ET) Visit occurs within 4 weeks of the last study drug dose or after withdrawal from study participation, whichever occurs last. Participants who discontinue from study drug early will be asked to continue to complete the remaining study assessments until study completion as long as such continued participation will not detrimentally affect the health, safety, and welfare of the participant per investigator determination.

[e] Additional observers identified after screening must be trained.

[f] A full PE will be performed at Week 73, Week 145, and the SC/ET visit. A brief PE will be performed at other visits. A full PE will include, at a minimum, general appearance (head, eyes, ears, nose, and throat) and an assessment of cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, musculoskeletal, and neurologic systems. A brief PE may be performed at the discretion of the investigator based on the participant's clinical condition and may include, but is not limited to, general appearance and an assessment of cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, musculoskeletal, and neurologic systems.

[g] It is recommended that urine samples are obtained as a first or second morning void. In the event of elevated urinary protein on a test result, a repeat urinalysis should be performed. This repeat urine sample must be performed in the morning at the first or second morning void to allow for accurate test results and may be performed by a home healthcare nurse.

[h] To be done by a local laboratory.

[i] Serum cortisol samples should be taken before study drug administration in the morning before 10:00 am. A home healthcare nurse may collect samples for participant convenience. If 2 results are low and abnormal, the participant will be asked to perform additional sampling for serum cortisol and a low-dose conventional ACTH stimulation test or the ACTH test method based on the clinical practice at the site.

[j] It is recommended that urine samples be obtained as a first or second morning void. Participants with a confirmed urine/albumin creatinine ratio of ≥ 100 mg/g should be referred to a nephrologist for consultation if results were within normal range at Baseline. Participants who had elevated results at Baseline followed by a confirmed subsequent increase of 100-200 mg/g from Baseline should also be referred to a nephrologist for consultation.

[k] To be done locally (if applicable). If urine pregnancy test is positive or equivocal, serum pregnancy test (central laboratory) must be done.

[l] Participants should record at home all food, beverages, special low-protein foods, and medical foods consumed during the 3 consecutive days preceding a study visit.

[m] Blood is collected before study drug administration (pre-dose) within 2 hours of dosing. Intensive PK sampling will be performed at Week 73 and at dose escalation visits in all participants. Samples will be taken at pre-dose (within 2 hours of dosing); at 2, 4, 8, and 12 hours (± 30 minutes) post-dose; and 24 hours (± 2 hours) post dose. The 24-hour sample will be taken prior to the next daily dose.

[n] Sample collection should be performed where allowed by country-specific regulations and EC/IRB.

[o] Blood is collected for blood Phe analysis after fasting a minimum of 2.5 hours.

[p] Immunogenicity assays include total anti-pegvaliase antibodies (TAb), anti-PAL IgG, anti-PAL IgM, anti-PEG IgM, anti-PEG IgG, and neutralizing antibodies (NAb). At the HRV, only anti-pegvaliase IgE will be assessed.

[q] Completed by parent/guardian. Assessments should be completed before other study visit assessments (eg, vital signs, blood draw for blood Phe, and study drug administration).

[r] Completed by participant. Assessments should be completed before other study visit assessments (eg, vital signs, blood draw for blood Phe, and study drug administration).

[s] Completed by the investigator or designee clinician. Assessments should be completed before other study visit assessments (eg, vital signs, blood draw for blood Phe, and study drug administration). Visits where CGIS will be assessed should take place in the hospital setting or clinic.

[t] Between scheduled clinic visits, the clinic staff will contact the participant via phone weekly to monitor if the participant is experiencing problems with drug administration, to ask about any AEs or concomitant medications, and to answer questions.

[u] AEs and concomitant medications should be noted whenever a participant is assessed by study personnel. All AEs and SAEs are collected until 4 weeks after the last study drug dose or the Study Completion Visit/Early Termination visit, whichever occurs last. If there is a severe (Grade 3) injection site reaction, the participant may be referred to a dermatologist for consultation and a skin biopsy (optional; if clinically indicated). If photographs of the severe injection site reaction are taken, they may be collected by the sponsor.

[v] Participants must be premedicated with an H1 antagonist, an H2 antagonist, and an antipyretic approximately 2 to 3 hours prior to each dose of study drug until they reach the Maintenance Phase, defined as when participants achieve blood Phe ≤ 600 µmol/L while they are on a stable dose of pegvaliase. Premedication may be considered during the Maintenance Phase at the investigator's discretion, eg, approximately 2 to 3 hours prior to study drug for 1 week upon reintroduction of study drug, upon resolution of an AE, following any dose interruption of ≥ 4 days, and for any dose increases in the Extension Phase. Participants may also be premedicated at any other time in the study per investigator discretion. When an NSAID is administered as a premedication, it should be given with food. For non-clinic visits, participants are asked about study drug administration.

Table 1.3.3: Schedule of Activities for Part 2 (Extension Phase) Weeks 153-215 (Participants Randomized to Pegvaliase Treatment Arm)

Assessment [a]	Study Week	153	157	161	165	169	173	177	181	185	189	193	197	201	205	209	213	215	HRV[b]	SC/ET[c]
	Study Day	1065	1093	1121	1149	1177	1205	1233	1261	1289	1317	1345	1373	1401	1429	1457	1485	1499		
	Window (Days)	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7		
Study drug observer training [d]																				
12 lead ECG																				X
Physical examination [e]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Weight	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Height	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
Vital signs	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Clinical safety laboratory tests [f]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
C-reactive protein	X		X		X		X		X		X		X		X		X	X	X	X
Erythrocyte sedimentation rate [g]																				X
Serum cortisol [h]	X									X										X
Complements C3 and C4	X		X		X		X		X	X	X		X		X		X	X	X	X
Tryptase																			X	
Urine albumin/creatinine ratio [i]	X		X		X		X		X		X		X		X		X	X	X	X
Urine N-methylhistamine																			X	
Urine pregnancy test [j]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
3-day diet diary [k]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
PK (plasma pegvaliase) [l]	X		X		X		X		X		X		X		X		X			X
Blood for exploratory analyses [m]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
Blood Phe, tyrosine [n]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Immunogenicity assessments [o]	X		X		X		X		X		X		X		X		X	X	X	X
ADHD RS-IV [p]		X			X			X			X			X			X			X
BRIEF2 [p]		X			X			X			X			X			X			X
PKU-SSIS [q]		X			X			X			X			X			X			X

	Study Week	153	157	161	165	169	173	177	181	185	189	193	197	201	205	209	213	215		
	Study Day	1065	1093	1121	1149	1177	1205	1233	1261	1289	1317	1345	1373	1401	1429	1457	1485	1499		
Assessment [a]	Window (Days)	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	HRV[b]	SC/ET[c]
PGIS [q]			X			X			X			X			X			X		X
PGIC [q]									X									X		X
CaGIS [p]			X			X			X			X			X			X		X
CaGIC [p]									X									X		X
CGIS [r]			X			X			X			X			X			X		X
Weekly Phone Call (non-visit weeks) [s]																				
Adverse events [t]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant medication [t]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Administer study drug [u]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		

ADHD RS-IV, attention deficit hyperactivity disorder rating scale IV; BRIEF2, Behavior Rating Inventory of Executive Function, Second Edition; ECG, electrocardiogram; eCRF, electronic Case Report Form; HRV, hypersensitivity reaction visit; PE, physical examination; PK, pharmacokinetics; PKU, phenylketonuria; NSAID, non-steroidal anti-inflammatory drug; SC/ET, Study Completion/Early Termination visit.

[a] Scheduled visits take place at the hospital setting (for all dose escalation visits and visits where the CGIS assessments will be performed), clinic, or, with the approval of the investigator or designee, virtually. For virtual visits the investigator/designee performs assessments while online with the participant, apart from specimen collections and vital signs, which are performed by home healthcare personnel. Non-clinic visits are via telephone. Assessments are performed pre-dose unless otherwise specified.

[b] If the investigator determines that the NCI-CTCAE Grade ≥ 3 hypersensitivity reaction is related to administration with study drug, the participant will be asked to return to the clinic within 24 hours of event onset for a hypersensitivity reaction visit (HRV). Sampling for anti-pegvaliase IgE should be performed 8 to 24 hours after event onset and before the next dose of study drug.

[c] Participants should perform the Study Completion (SC) Visit 4 weeks after the last dose of study drug or after completion of study participation, whichever occurs last. For participants who terminate from the study early, the Early Termination (ET) Visit occurs within 4 weeks of the last study drug dose or after withdrawal from study participation, whichever occurs last. Participants who discontinue from study drug early will be asked to continue to complete the remaining study assessments until study completion as long as such continued participation will not detrimentally affect the health, safety, and welfare of the participant per investigator determination.

[d] Additional observers identified after screening must be trained.

[e] A full PE will be performed at the SC/ET visit. A brief PE will be performed at other visits. A full PE will include, at a minimum, general appearance (head, eyes, ears, nose, and throat) and an assessment of cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, musculoskeletal, and neurologic systems. A brief PE may

be performed at the discretion of the investigator based on the participant's clinical condition and may include, but is not limited to, general appearance and an assessment of cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, musculoskeletal, and neurologic systems.

[f] It is recommended that urine samples are obtained as a first or second morning void. In the event of elevated urinary protein on a test result, a repeat urinalysis should be performed. This repeat urine sample must be performed in the morning at the first or second morning void to allow for accurate test results and may be performed by a home healthcare nurse.

[g] To be done by a local laboratory.

[h] Serum cortisol samples should be taken before study drug administration in the morning before 10:00 am. A home healthcare nurse may collect samples for participant convenience. If 2 results are low and abnormal, the participant will be asked to perform additional sampling for serum cortisol and a low-dose conventional ACTH stimulation test or the ACTH test method based on the clinical practice at the site.

[i] It is recommended that urine samples be obtained as a first or second morning void. Participants with a confirmed urine/albumin creatinine ratio of ≥ 100 mg/g should be referred to a nephrologist for consultation if results were within normal range at Baseline. Participants who had elevated results at Baseline followed by a confirmed subsequent increase of 100-200 mg/g from Baseline should also be referred to a nephrologist for consultation.

[j] To be done locally (if applicable). If urine pregnancy test is positive or equivocal, serum pregnancy test (central laboratory) must be done.

[k] Participants should record at home all food, beverages, special low-protein foods, and medical foods consumed during the 3 consecutive days preceding a study visit. No change in protein (dietary and medical food) intake is allowed in Part 1.

[l] Blood is collected before study drug administration (pre-dose) within 2 hours of dosing. Intensive PK sampling will be performed at dose escalation visits in all participants. Samples will be taken at pre-dose (within 2 hours of dosing); at 2, 4, 8, and 12 hours (± 30 minutes) post-dose; and 24 hours (± 2 hours) post dose. The 24-hour sample will be taken prior to the next daily dose.

[m] Sample collection should be performed where allowed by country-specific regulations and EC/IRB.

[n] Blood is collected for blood Phe analysis after fasting a minimum of 2.5 hours.

[o] Immunogenicity assays include total anti-pegvaliase antibodies (TAb), anti-PAL IgG, anti-PAL IgM, anti-PEG IgM, anti-PEG IgG, and neutralizing antibodies (NAb). At the HRV, only anti-pegvaliase IgE will be assessed.

[p] Completed by parent/guardian. Assessments should be completed before other study visit assessments (eg, vital signs, blood draw for blood Phe, and study drug administration).

[q] Completed by participant. Assessments should be completed before other study visit assessments (eg, vital signs, blood draw for blood Phe, and study drug administration).

[r] Completed by the investigator or designee clinician. Assessments should be completed before other study visit assessments (eg, vital signs, blood draw for blood Phe, and study drug administration). Visits where CGIS will be assessed should take place in the hospital setting or clinic.

[s] Between scheduled clinic visits, the clinic staff will contact the participant weekly to monitor if the participant is experiencing problems with drug administration, to ask about any AEs or concomitant medications, and to answer questions.

[t] AEs and concomitant medications should be noted whenever a participant is assessed by study personnel. All AEs and SAEs are collected until 4 weeks after the last study drug dose or the Study Completion Visit/Early Termination visit, whichever occurs last. If there is a severe (Grade 3) injection site reaction, the participant may be referred to a dermatologist for consultation and a skin biopsy (optional; if clinically indicated). If photographs of the severe injection site reaction are taken, they may be collected by the sponsor.

[u] Participants must be premedicated with an H1 antagonist, and an H2 antagonist, and an antipyretic approximately 2 to 3 hours prior to each dose of study drug until they reach the Maintenance Phase, defined as when participants achieve blood Phe ≤ 600 µmol/L while they are on a stable dose of pegvaliase. Premedication may be considered during the Maintenance Phase at the investigator's discretion, eg, approximately 2 to 3 hours prior to study drug for 1 week upon reintroduction of study drug, upon resolution of an AE, following any dose interruption of ≥ 4 days, and for any dose increases in the Extension Phase. Participants may also be premedicated at any other time in the study per investigator discretion. When an NSAID is administered as a premedication, it should be given with food. For non-clinic visits, participants are asked about study drug administration.

Table 1.3.4: Schedule of Activities for Part 1 (Participants Randomized to Diet-Only Treatment Arm)

Assessment [a]	Study Week	Screen[b]	1	2	3	4	5	6	7	8	9	13	17	21	25	29	33	37	41	45	49	53	57	61	65	69	73	SC/ET[c]
	Study Day	-28/-1	1	8	15	22	29	36	43	50	57	85	113	141	169	197	225	253	281	309	337	365	393	421	449	477	505	
	Window (Days)			±3	±3	±3	±3	±3	±3	±3	±3	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±3	
Informed consent		X																										
Drug administration training [d]																											X	
Study drug observer training [e]																											X	
Demographics		X																										
Medical history (allergy, PKU) [f]		X																										
HIV, hepatitis B and C screens [g]		X																										
PAH genotyping [h]			X																									
12 lead ECG		X	X																								X	X
Physical examination [i]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Weight		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Height		X	X				X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital signs		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Clinical safety laboratory tests [j]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
C-reactive protein		X	X								X		X		X		X		X		X		X		X		X	X
Erythrocyte sedimentation rate [k]		X	X																								X	X
Serum cortisol [l]			X												X						X						X	X
Complements C3 and C4			X				X				X		X		X		X		X		X		X		X		X	X
Tryptase			X																								X	
Urine albumin/creatinine ratio [m]			X								X		X		X		X		X		X		X		X		X	X
Urine pregnancy test [n]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
3-day diet diary [o]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Blood for exploratory analyses [h]			X				X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Blood Phe, tyrosine [p]		X [b]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
ADHD RS-IV [q]			X									X			X			X			X			X			X	X

Assessment [a]	Study Week	Screen[b]	1	2	3	4	5	6	7	8	9	13	17	21	25	29	33	37	41	45	49	53	57	61	65	69	73	SC/ET[c]
	Study Day	-28/-1	1	8	15	22	29	36	43	50	57	85	113	141	169	197	225	253	281	309	337	365	393	421	449	477	505	
	Window (Days)			±3	±3	±3	±3	±3	±3	±3	±3	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±3	
BRIEF2 [q]			X									X			X			X			X			X			X	X
PKU-SSIS [r,v]			X									X			X			X			X			X			X	X
PGIS [r,v]			X									X			X			X			X			X			X	X
PGIC [r]																		X									X	X
CaGIS [q,v]			X									X			X			X			X			X			X	X
CaGIC [q]																		X									X	X
CGIS [s,v]			X									X			X			X			X			X			X	X
Weekly Phone Call (non-visit weeks) [t]																												
Adverse events [u]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant medication [u]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

ADHD RS-IV, attention deficit hyperactivity disorder rating scale IV; BRIEF2, Behavior Rating Inventory of Executive Function, Second Edition; EC/IRB, Ethics Committee/Institutional Review Board; ECG, electrocardiogram; eCRF, electronic Case Report Form; HRV, hypersensitivity reaction visit; PAH, phenylalanine hydroxylase; PE, physical examination; PK, pharmacokinetics; PKU, phenylketonuria; SC/ET, Study Completion/Early Termination visit.

[a] Scheduled visits take place at the clinic or, with the approval of the investigator or designee, virtually. For virtual visits the investigator/designee performs assessments while online with the participant, apart from specimen collections and vital signs, which are performed by home healthcare personnel. Non-clinic visits are via telephone.

[b] After written informed consent, screening assessments should be performed within 28 days prior to Part 1, Day 1. Participants will be assessed for blood Phe concentration during Screening/Run-in with 2 measurements 7 to 10 days apart. These 2 blood Phe measurements should be scheduled to occur: (1) in the Day -25 to Day -23 window; and (2) 7 to 10 days later (ie, in the Day -18 to Day -13 window).

[c] Participants should perform the Study Completion (SC) Visit 4 weeks after the last dose of study drug or after completion of study participation, whichever occurs last. For participants who terminate from the study early, the Early Termination (ET) Visit occurs within 4 weeks of the last study drug dose or after withdrawal from study participation, whichever occurs last. Participants who discontinue from study drug early will be asked to continue to complete the remaining study assessments until study completion as long as such continued participation will not detrimentally affect the health, safety, and welfare of the participant per investigator determination.

[d] Once participant/caregiver competence is documented, the participant/caregiver administers study drug.

[e] Must be completed prior to administration of the first dose of study drug in Part 2. Additional observers identified after Screening must be trained.

[f] PKU history includes highest blood Phe (and age).

[g] Participants with documented negative results within the last 4 weeks do not need to be retested.

[h] Sample collection should be performed where allowed by country-specific regulations and EC/IRB.

[i] A full PE will be performed at Screening, Baseline, Week 73, and the SC/ET visit. A brief PE will be performed at other visits. A full PE will include, at a minimum, general appearance (head, eyes, ears, nose, and throat), and an assessment of cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, musculoskeletal, and neurologic systems. A brief PE may be performed at the discretion of the investigator based on the participant's clinical condition and may include, but is not limited to, general appearance and an assessment of cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, musculoskeletal, and neurologic systems.

[j] It is recommended that urine samples are obtained as a first or second morning void. In the event of elevated urinary protein on a test result, a repeat urinalysis should be performed. This repeat urine sample must be performed in the morning at the first or second morning void to allow for accurate test results and may be performed by a home healthcare nurse.

[k] To be done by a local laboratory.

[l] Serum cortisol samples should be taken before study drug administration in the morning before 10:00 am. A home healthcare nurse may collect samples for participant convenience. If 2 results are low and abnormal, the participant will be asked to perform additional sampling for serum cortisol and a low-dose conventional ACTH stimulation test or the ACTH test method based on the clinical practice at the site.

[m] It is recommended that urine samples be obtained as a first or second morning void. Participants with a confirmed urine/albumin creatinine ratio of ≥ 100 mg/g should be referred to a nephrologist for consultation if results were within normal range at Baseline. Participants who had elevated results at Baseline followed by a confirmed subsequent increase of 100-200 mg/g from Baseline should also be referred to a nephrologist for consultation.

[n] To be done locally (if applicable). If urine pregnancy test is positive or equivocal, serum pregnancy test (central laboratory) must be done.

[o] Participants should record at home all food, beverages, special low-protein foods, and medical foods consumed during the 3 consecutive days preceding a study visit. No change in protein (dietary and medical food) intake is allowed in Part 1. During Screening/Run-in, 3-day diet diary assessments are performed 3 times, each time covering the 3-day periods prior to blood Phe assessments (and collected on the day of those assessments). The 3 Screening/Run-in blood Phe assessments are scheduled to occur (1) at Day -25 to Day -23; (2) 7 to 10 days later (ie, in the Day -18 to Day -13 window); and (3) at Day 1 pre-dose. Thus, the 3 day diet assessments will assess diet for (1) the 3 days within the Day -28 to Day -23 window immediately prior to the first blood Phe assessment; (2) the 3 days within the Day -21 to Day -15 window immediately prior to the second blood Phe assessment; and (3) for Day -3 to Day 1.

[p] Blood is collected for blood Phe analysis after fasting a minimum of 2.5 hours. In addition to the blood draws for blood Phe analysis collected in the clinic and shown in the SoA, there are 3 required blood collections that may be drawn either at the clinic or elsewhere (eg, at home). Diet diary data collection must be done prior to all Phe collections.

[q] Completed by parent/guardian. Assessments should be completed before other study visit assessments (eg, vital signs, blood draw for blood Phe, and study drug administration).

[r] Completed by participant. Assessments should be completed before other study visit assessments (eg, vital signs, blood draw for blood Phe, and study drug administration).

[s] Completed by the investigator or designee clinician. Assessments should be completed before other study visit assessments (eg, vital signs, blood draw for blood Phe, and study drug administration). Visits where CGIS will be assessed should take place in the hospital setting or clinic.

[t] Between scheduled clinic visits, the clinic staff will contact the participant weekly to ask about any AEs or concomitant medications, and to answer questions.

[u] AEs and concomitant medications should be noted whenever a participant is assessed by study personnel. Following signed informed consent all AEs and SAEs are collected throughout Part 1 or until the Early Termination Visit, whichever occurs last.

[v] Initial assessments of the PKU-SSIS, PGIS, CaGIS, and CGIS will be at the first visit following implementation/adoption of Protocol Amendment 2.

Table 1.3.5: Schedule of Activities for Part 2 (Primary Treatment Phase – Weeks 73-145)
(Pegvaliase Treatment for Participants Randomized to Diet-Only Treatment Arm)

Assessment [a]	Study Week	73[b]	74	75	76	77	78	79	80	81	83	85	87	89	91	93	97	101	105	109	113	117	121	125	129	133	137	141	145	HRV[c]	SC/ET[d]
	Study Day	**505**	**512**	**519**	**526**	**533**	**540**	**547**	**554**	**561**	**575**	**589**	**603**	**617**	**631**	**645**	**673**	**701**	**729**	**757**	**785**	**813**	**841**	**869**	**897**	**925**	**953**	**981**	**1009**		
	Window (Days)	**±3**	**±3**	**±3**	**±3**	**±3**	**±3**	**±3**	**±3**	**±3**	**±3**	**±7**	**±3**	**±7**	**±3**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**		
Drug administration training [e]		X																													
Study drug observer training [f]																															
12 lead ECG		X																													X
Physical examination [g]		X	X	X	X	X	X	X	X	X		X		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Weight		X	X	X	X	X	X	X	X	X		X		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Height		X				X				X		X		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
Vital signs		X	X	X	X	X	X	X	X	X		X		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Clinical safety laboratory tests [h]		X	X	X	X	X	X	X	X	X		X		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
C-reactive protein		X								X				X			X		X		X		X		X		X		X	X	X
Erythrocyte sedimentation rate [i]		X																													X
Serum cortisol [j]		X															X						X						X		X
Complements C3 and C4		X				X				X		X		X		X	X		X		X		X		X		X		X	X	X
Tryptase		X																												X	
Urine albumin/creatinine ratio [k]		X								X				X			X		X		X		X		X		X		X	X	X
Urine N-methylhistamine		X																												X	
Urine pregnancy test [l]		X	X	X	X	X	X	X	X	X		X		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
3-day diet diary [m]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
PK (plasma pegvaliase) [n]		X				X				X		X		X		X	X		X		X		X		X		X		X		X

Assessment [a]	Study Week	73[b]	74	75	76	77	78	79	80	81	83	85	87	89	91	93	97	101	105	109	113	117	121	125	129	133	137	141	145	HRV[c]	SC/ET[d]
	Study Day	505	512	519	526	533	540	547	554	561	575	589	603	617	631	645	673	701	729	757	785	813	841	869	897	925	953	981	1009		
	Window (Days)	±3	±3	±3	±3	±3	±3	±3	±3	±3	±3	±7	±3	±7	±3	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7		
Blood for exploratory analyses [o]		X				X				X		X		X		X	X	X	X	X	X	X	X	X	X	X	X	X		X
Blood Phe, tyrosine [p]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Immunogenicity assessments [q]		X	X	X	X	X	X	X	X	X		X		X		X	X		X		X		X		X		X		X	X	X
ADHD RS-IV [r]		X										X					X			X			X			X			X		X
BRIEF2 [r]		X										X					X			X			X			X			X		X
PKU-SSIS [s]		X										X					X			X			X			X			X		X
PGIS [s]		X										X					X			X			X			X			X		X
PGIC [s]		X																		X									X		X
CaGIS [r]		X										X					X			X			X			X			X		X
CaGIC [r]		X																		X									X		X
CGIS [t]		X										X					X			X			X			X			X		X
Weekly Phone Call (non-visit weeks) [u]																															
Adverse events [v]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant medication [v]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Administer study drug [w]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		

ADHD RS-IV, attention deficit hyperactivity disorder rating scale IV; BRIEF2, Behavior Rating Inventory of Executive Function, Second Edition; ECG, electrocardiogram; eCRF, electronic Case Report Form; HRV, hypersensitivity reaction visit; NSAID, non-steroidal anti-inflammatory drug; PE, physical examination; PK, pharmacokinetics; PKU, phenylketonuria; SC/ET, Study Completion/Early Termination visit.

[a] Scheduled visits take place at the hospital setting (for all dosing visits for the first 8 weeks, and for all dose escalation visits), clinic, or, with the approval of the investigator or designee, virtually. For virtual visits the investigator/designee performs assessments while online with the participant, apart from specimen collections and vital signs, which are performed by home healthcare personnel. Non-clinic visits are via telephone. Assessments are performed pre-dose unless otherwise specified.

[b] For Cohort B control participants, the Week 73 visit marks both the end of Part 1, when their PKU is treated with diet alone, and the beginning of Part 2, when they receive pegvaliase. The Week 73 pre-dose assessments constituting the end of Part 1 are shown in the Week 73 column of Table 1.3.4. These same assessments, used as baseline assessments for Part 2, are shown in the Week 73 column of Table 1.3.5, along with the administration of study drug that marks the beginning of Part 2.

[c] If the investigator determines that the NCI-CTCAE Grade ≥ 3 hypersensitivity reaction is related to administration with study drug, the participant will be asked to return to the clinic within 24 hours of event onset for a hypersensitivity reaction visit (HRV). Sampling for anti-pegvaliase IgE should be performed 8 to 24 hours after event onset and before the next dose of study drug.

[d] Participants should perform the Study Completion (SC) Visit 4 weeks after the last dose of study drug or after completion of study participation, whichever occurs last. For participants who terminate from the study early, the Early Termination (ET) Visit occurs within 4 weeks of the last study drug dose or after withdrawal from study participation, whichever occurs last. Participants who discontinue from study drug early will be asked to continue to complete the remaining study assessments until study completion as long as such continued participation will not detrimentally affect the health, safety, and welfare of the participant per investigator determination.

[e] Once participant/caregiver competence is documented, the participant/caregiver administers study drug daily.

[f] Must be completed prior to administration of the first dose of study drug. Additional observers identified after screening must be trained.

[g] A full PE will be performed at Week 73, Week 145, and the SC/ET visit. A brief PE will be performed at other visits. A full physical examination will include, at a minimum, general appearance (head, eyes, ears, nose, and throat), and an assessment of cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, musculoskeletal, and neurologic systems. A brief physical examination may be performed at the discretion of the investigator based on the participant's clinical condition and may include, but is not limited to, general appearance and an assessment of cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, musculoskeletal, and neurologic systems.

[h] It is recommended that urine samples are obtained as a first or second morning void. In the event of elevated urinary protein on a test result, a repeat urinalysis should be performed. This repeat urine sample must be performed in the morning at the first or second morning void to allow for accurate test results and may be performed by a home healthcare nurse.

[i] To be done by a local laboratory.

[j] Serum cortisol samples should be taken before study drug administration in the morning before 10:00 am. A home healthcare nurse may collect samples for participant convenience. If 2 results are low and abnormal, the participant will be asked to perform additional sampling for serum cortisol and a low-dose conventional ACTH stimulation test or the ACTH test method based on the clinical practice at the site.

[k] It is recommended that urine samples be obtained as a first or second morning void. Participants with a confirmed urine/albumin creatinine ratio of ≥ 100 mg/g should be referred to a nephrologist for consultation if results were within normal range at Baseline. Participants who had elevated results at Baseline followed by a confirmed subsequent increase of 100-200 mg/g from Baseline should also be referred to a nephrologist for consultation.

[l] To be done locally (if applicable). If urine pregnancy test is positive or equivocal, serum pregnancy test (central laboratory) must be done.

[m] Participants should record at home all food, beverages, special low-protein foods, and medical foods consumed during the 3 consecutive days preceding a study visit. No change in protein (dietary and medical food) intake is allowed in Part 1.

[n] Blood is collected before study drug administration (pre-dose) within 2 hours of dosing. Intensive PK sampling will be performed at Week 145 and at dose escalation visits in all participants. Samples will be taken at pre-dose (within 2 hours of dosing); at 2, 4, 8, and 12 hours (± 30 minutes) post-dose; and 24 hours (± 2 hours) post dose. The 24-hour sample will be taken prior to the next daily dose.

[o] Sample collection should be performed where allowed by country-specific regulations and EC/IRB.

[p] Blood is collected for blood Phe analysis after fasting a minimum of 2.5 hours. In addition to the blood draws for blood Phe analysis collected in the clinic and shown in the SoA, there are 5 required blood collections that may be drawn either at the clinic or elsewhere (eg, at home). These are to occur at Weeks 83, 87, and 91, and 2 weeks prior to the planned escalations to 40 and 60 mg/day. Diet diary data collection must be done prior to all Phe collections.

[q] Immunogenicity assays include total anti-pegvaliase antibodies (TAb), anti-PAL IgG, anti-PAL IgM, anti-PEG IgM, anti-PEG IgG, and neutralizing antibodies (NAb). At the HRV, only anti-pegvaliase IgE will be assessed.

[r] Completed by parent/guardian. Assessments should be completed before other study visit assessments (eg, vital signs, blood draw for blood Phe, and study drug administration).

[s] Completed by participant. Assessments should be completed before other study visit assessments (eg, vital signs, blood draw for blood Phe, and study drug administration).

[t] Completed by the investigator or designee clinician. Assessments should be completed before other study visit assessments (eg, vital signs, blood draw for blood Phe, and study drug administration). Visits where CGIS will be assessed should take place in the hospital setting or clinic.

[u] Between scheduled clinic visits, the clinic staff will contact the participant weekly to monitor if the participant is experiencing problems with drug administration, to ask about any AEs or concomitant medications, and to answer questions.

[v] AEs and concomitant medications should be noted whenever a participant is assessed by study personnel. Beginning with start of study treatment, All AEs and SAEs are collected until 4 weeks after the last study drug dose or the Study Completion Visit/Early Termination visit, whichever occurs last. If there is a severe (Grade 3) injection site reaction, the participant may be referred to a dermatologist for consultation and a skin biopsy (optional; if clinically indicated). If photographs of the severe injection site reaction are taken, they may be collected by the sponsor.

[w] Participants must be premedicated with an H1 antagonist, an H2 antagonist, and an antipyretic approximately 2 to 3 hours prior to each dose of study drug until they reach the Maintenance Phase, defined as when participants achieve blood Phe ≤ 600 µmol/L while they are on a stable dose of pegvaliase. Premedication may be considered during the Maintenance Phase at the investigator's discretion, eg, approximately 2 to 3 hours prior to study drug for 1 week upon reintroduction of study drug, upon resolution of an AE, following any dose interruption of ≥ 4 days, and for any dose increases in the Extension Phase. Participants may also be premedicated at any other time in the study per investigator discretion. When an NSAID is administered as a premedication, it should be given with food. For non-clinic visits, participants are asked about study drug administration.

Table 1.3.6: Schedule of Activities for Part 2 (Extension Phase – Weeks 149-215)
(Pegvaliase Treatment for Participants Randomized to Diet-Only Treatment Arm)

Assessment [a]	Study Week	145[b]	149	153	157	161	165	169	173	177	181	185	189	193	197	201	205	209	213	215	HRV[c]	SC/ET[d]
	Study Day	1009	1037	1065	1093	1121	1149	1177	1205	1233	1261	1289	1317	1345	1373	1401	1429	1457	1485	1499		
	Window (Days)	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7		
Study drug observer training [e]																						
12 lead ECG																						X
Physical examination [f]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Weight	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Height	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
Vital signs	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Clinical safety laboratory tests [g]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
C-reactive protein	X		X		X		X		X		X		X		X		X		X	X	X	X
Erythrocyte sedimentation rate [h]																						X
Serum cortisol [i]	X						X						X						X			X
Complements C3 and C4	X		X		X		X		X		X		X		X		X		X	X	X	X
Tryptase																					X	
Urine albumin/creatinine ratio [j]	X		X		X		X		X		X		X		X		X		X	X	X	X
Urine N-methylhistamine																					X	
Urine pregnancy test [k]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
3-day diet diary [l]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
PK (plasma pegvaliase) [m]	X		X		X		X		X		X		X		X		X		X			X
Blood for exploratory analyses [n]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
Blood Phe, tyrosine [o]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Immunogenicity assessments [p]	X		X		X		X		X		X		X		X		X		X	X	X	X
ADHD RS-IV [q]	X			X			X			X			X			X			X			X
BRIEF2 [q]	X			X			X			X			X			X			X			X
PKU-SSIS [r]	X			X			X			X			X			X			X			X

	Study Week	145[b]	149	153	157	161	165	169	173	177	181	185	189	193	197	201	205	209	213	215		
	Study Day	1009	1037	1065	1093	1121	1149	1177	1205	1233	1261	1289	1317	1345	1373	1401	1429	1457	1485	1499		
Assessment [a]	Window (Days)	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	HRV[c]	SC/ET[d]
PGIS [r]		X			X			X			X			X			X			X		X
PGIC [r]		X									X									X		X
CaGIS [q]		X			X			X			X			X			X			X		X
CaGIC [q]		X									X									X		X
CGIS [s]		X			X			X			X			X			X			X		X
Weekly Phone Call (non-visit weeks) [t]																						
Adverse events [u]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant medication [u]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Administer study drug [v]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		

ADHD RS-IV, attention deficit hyperactivity disorder rating scale IV; BRIEF2, Behavior Rating Inventory of Executive Function, Second Edition; ECG, electrocardiogram; eCRF, electronic Case Report Form; HRV, hypersensitivity reaction visit; NSAID, non-steroidal anti-inflammatory drug; PE, physical examination; PK, pharmacokinetics; PKU, phenylketonuria; SC/ET, Study Completion/Early Termination visit.

[a] Scheduled visits take place at the hospital setting (for all dosing visits for the first 8 weeks, and for all dose escalation visits), clinic, or, with the approval of the investigator or designee, virtually. For virtual visits the investigator/designee performs assessments while online with the participant, apart from specimen collections and vital signs, which are performed by home healthcare personnel. Non-clinic visits are via telephone. Assessments are performed pre-dose unless otherwise specified.

[b] The Week 145 visit marks both the end of the Primary Treatment Phase and the beginning of the Extension Phase. The pre-dose assessments constituting the end of the Primary Treatment Phase and the administration of study drug constituting the beginning of the Extension Phase are shown in the Week 73 columns of both Table 1.3.5 and Table 1.3.6.

[c] If the investigator determines that the NCI-CTCAE Grade ≥ 3 hypersensitivity reaction is related to administration with study drug, the participant will be asked to return to the clinic within 24 hours of event onset for a hypersensitivity reaction visit (HRV). Sampling for anti-pegvaliase IgE should be performed 8 to 24 hours after event onset and before the next dose of study drug.

[d] Participants should perform the Study Completion (SC) Visit 4 weeks after the last dose of study drug or after completion of study participation, whichever occurs last. For participants who terminate from the study early, the Early Termination (ET) Visit occurs within 4 weeks of the last study drug dose or after withdrawal from study participation, whichever occurs last. Participants who discontinue from study drug early will be asked to continue to complete the remaining study assessments until study completion as long as such continued participation will not detrimentally affect the health, safety, and welfare of the participant per investigator determination.

[e] Additional observers identified after screening must be trained.

[f] A full PE will be performed at the SC/ET visit. A brief PE will be performed at other visits. A full physical examination will include, at a minimum, general appearance (head, eyes, ears, nose, and throat), and an assessment of cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, musculoskeletal, and neurologic systems. A brief physical examination may be performed at the discretion of the investigator based on the participant's clinical condition and may include, but is not limited to, general appearance and an assessment of cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, musculoskeletal, and neurologic systems.

[g] It is recommended that urine samples are obtained as a first or second morning void. In the event of elevated urinary protein on a test result, a repeat urinalysis should be performed. This repeat urine sample must be performed in the morning at the first or second morning void to allow for accurate test results and may be performed by a home healthcare nurse.

[h] To be done by a local laboratory.

[i] Serum cortisol samples should be taken before study drug administration in the morning before 10:00 am. A home healthcare nurse may collect samples for participant convenience. If 2 results are low and abnormal, the participant will be asked to perform additional sampling for serum cortisol and a low-dose conventional ACTH stimulation test or the ACTH test method based on the clinical practice at the site.

[j] It is recommended that urine samples be obtained as a first or second morning void. Participants with a confirmed urine/albumin creatinine ratio of ≥ 100 mg/g should be referred to a nephrologist for consultation if results were within normal range at Baseline. Participants who had elevated results at Baseline followed by a confirmed subsequent increase of 100-200 mg/g from Baseline should also be referred to a nephrologist for consultation.

[k] To be done locally (if applicable). If urine pregnancy test is positive or equivocal, serum pregnancy test (central laboratory) must be done.

[l] Participants should record at home all food, beverages, special low-protein foods, and medical foods consumed during the 3 consecutive days preceding a study visit. No change in protein (dietary and medical food) intake is allowed in Part 1.

[m] Blood is collected before study drug administration (pre-dose) within 2 hours of dosing. Intensive PK sampling will be performed at Week 145 and at dose escalation visits in all participants. Samples will be taken at pre-dose (within 2 hours of dosing), at 2, 4, 8, and 12 hours (± 30 minutes) post-dose; and 24 hours (± 2 hours) post dose. The 24-hour sample will be taken prior to the next daily dose.

[n] Sample collection should be performed where allowed by country-specific regulations and EC/IRB.

[o] Blood is collected for blood Phe analysis after fasting a minimum of 2.5 hours. In addition to the blood draws for blood Phe analysis collected in the clinic and shown in the SoA, there are 5 required blood collections that may be drawn either at the clinic or elsewhere (eg, at home). These are to occur at Weeks 83, 87, and 91, and 2 weeks prior to the planned escalations to 40 and 60 mg/day. Diet diary data collection must be done prior to all Phe collections.

[p] Immunogenicity assays include total anti-pegvaliase antibodies (TAb), anti-PAL IgG, anti-PAL IgM, anti-PEG IgM, anti-PEG IgG, and neutralizing antibodies (NAb). At the HRV, only anti-pegvaliase IgE will be assessed.

[q] Completed by parent/guardian. Assessments should be completed before other study visit assessments (e.g., vital signs, blood draw for blood Phe, and study drug administration).

[r] Completed by participant. Assessments should be completed before other study visit assessments (e.g., vital signs, blood draw for blood Phe, and study drug administration).

[s] Completed by the investigator or designee clinician. Assessments should be completed before other study visit assessments (e.g., vital signs, blood draw for blood Phe, and study drug administration). Visits where CGIS will be assessed should take place in the hospital setting or clinic.

[t] Between scheduled visits, the clinic staff will contact the participant weekly to monitor if the participant is experiencing problems with drug administration, to ask about any AEs or concomitant medications, and to answer questions.

[u] AEs and concomitant medications should be noted whenever a participant is assessed by study personnel. Beginning with start of study treatment, All AEs and SAEs are collected until 4 weeks after the last study drug dose or the Study Completion Visit/Early Termination visit, whichever occurs last. If there is a severe (Grade 3) injection site reaction, the participant may be referred to a dermatologist for consultation and a skin biopsy (optional; if clinically indicated). If photographs of the severe injection site reaction are taken, they may be collected by the sponsor.

[v] Participants must be premedicated with an H1 antagonist, and an H2 antagonist, and an antipyretic approximately 2 to 3 hours prior to each dose of study drug until they reach the Maintenance Phase, defined as when participants achieve blood Phe ≤ 600 µmol/L while they are on a stable dose of pegvaliase. Premedication may be considered during the Maintenance Phase at the investigator's discretion, eg, approximately 2 to 3 hours prior to study drug for 1 week upon reintroduction of study drug, upon resolution of an AE, following any dose interruption of ≥ 4 days, and for any dose increases in the Extension Phase. Participants may also be premedicated at any other time in the study per investigator discretion. When an NSAID is administered as a premedication, it should be given with food. For non-clinic visits, participants are asked about study drug administration.

2 INTRODUCTION

Phenylketonuria (PKU, OMIM 261600) is a rare autosomal recessive genetic disorder that is characterized by an inability of the body to break down the amino acid, phenylalanine (Phe), and is caused by mutations in the gene encoding phenylalanine hydroxylase (PAH). PAH catalyzes the conversion of the essential amino acid Phe to tyrosine, and this enzymatic activity is facilitated by tetrahydrobiopterin (BH4). PAH deficiency results in abnormally elevated concentrations of Phe. The estimated incidence of PKU is 1 in 15,000 births in the United States (US) (NIHCDP 2001).

The pathophysiology of PKU is well understood. A substantial body of evidence confirms that elevated Phe is directly toxic to cells in the brain, where it causes inhibition of protein synthesis in humans and animals (Wall 1990), affects the normal morphology of myelinating proteins (Dyer 1996), and leads to arrested or delayed development of dendrites and synapses in the cerebral cortex (Huttenlocher 2000). Neuropathology in PKU has been shown to derive from oxidative stress, impaired neurotransmitter metabolism, impaired protein synthesis and lipid metabolism, and brain energy metabolism alterations (Schuck 2015). The neurotoxicity of elevated Phe is a direct effect of Phe itself (Kaufman 1989). No abnormalities in Phe metabolites are observed in patients with PKU and normal Phe metabolites are toxic only at concentrations that are much higher than those reported in individuals with PKU.

With newborn screening followed by Medical Nutritional Therapy (MNT), the most severe manifestation of the disease, mental retardation, is prevented. Phe restriction with MNT involves consumption of semisynthetic medical foods that provide a source of Phe-free protein, modified low-protein foods, a small amount of natural protein (to provide required amounts of Phe), and other supplements (eg, vitamins and minerals) to correct nutritional deficiencies that can result from Phe restriction. Without highly restricted Phe intake and MNT, individuals with PKU quickly reach and sustain toxic concentrations of blood Phe. Individuals with classic PKU may have a complete absence or profound deficiency of PAH and typically show very high elevations of blood Phe (≥ 1200 µmol/L).

Historically, it was recommended that adherence to the Phe-restricted diet could end after age 6, but later studies have shown that patients who maintained Phe control longer had better outcomes in neuropsychological performance and areas of attention and speed of processing (Cerone 1999; Channon 2007; Rey 1996). A robust body of research suggests that early blood Phe control through dietary management is imperative in PKU populations, and that adult intelligence quotient (IQ) levels are correlated with the duration and vigilance of dietary management in childhood and adolescence (Pietz 1997; Smith 2000; Stemerdink 2000; Waisbren 1999).

To be optimally effective, a PKU diet needs to be started immediately after birth and continued for life. The American College of Medical Genetics and Genomics (ACMG) practice guidelines for PKU recommend life-long management and maintenance of metabolic control as essential to optimal functioning of individuals with PKU, with a goal of maintaining blood Phe concentrations ≤ 360 µmol/L (Vockley 2014). Similar European guidelines for the management of PKU recommend treatment target Phe levels of ≤ 600 µmol/L for patients older than 12 years (van Spronsen 2017).

Compliance with diet is a significant component of disease management, but life-long dietary compliance can be extremely difficult. A study conducted over 6 years at 4 treatment centers showed that dietary compliance in PKU populations was found to decline with age. Specifically, during infancy and in pre-school aged children, compliance with restrictive PKU diet is high because their diet is carefully managed by a parent and nutritionist. However, when PKU children start school, their blood Phe levels increase because of changes in socialization and changes in their palate, making compliance to a Phe-restricted diet more challenging (Walter 2002; Walter 2004). As children age, they become more likely to find formula and synthetic low-Phe foods distasteful and they become exposed to a broader range of food and peer pressure, both of which encourage noncompliance (MacDonald 2010). Adolescents/young adults with PKU are at particular risk for non-compliance with MNT as they transition away from childhood when parental oversight of dietary control decreases (Brown 2016). In addition, adolescents/young adults who have stopped MNT and attempt to resume a Phe-restricted diet into adulthood have difficulty maintaining the diet, with 31% of patients no longer maintaining dietary control after 10 months of restarting (Schuett 1985).

Therefore, despite the best efforts of the medical community and patients, life-long therapy with MNT with Phe restriction is not sustainable nor feasible as a treatment option for the majority of patients with PKU (Walter 2002; Burton 2010; Aoki 2007). The inability to adhere to MNT results in uncontrolled blood Phe levels associated with executive dysfunction, depression, and a variety of behavioral and psychiatric problems (Moyle 2007; Pietz 1997; Smith 2000; Waisbren 1999). Adolescents with PKU with poor metabolic control represent a patient population in great need of additional therapeutic options.

BioMarin has developed pegvaliase as a subcutaneous enzyme substitution treatment to reduce blood Phe levels in adult patients with PKU. Enzyme replacement therapy with PAH is not feasible due to the instability of PAH in plasma and the difficulty with efficiently delivering an intact, active form of the enzyme to the liver (Harding 2003; Kim 2004). Pegvaliase is a genetically modified phenylalanine ammonia lyase (PAL) enzyme product of

the cyanobacterium *Anabaena variabilis*, which is PEGylated to decrease immunogenicity and increase half-life. Unlike PAH, the PAL enzyme is active in plasma and, independently of cofactor BH4 which is required for PAH activity, catalyzes Phe to trans-cinnamic acid (t-CA) and ammonia, which are metabolized in the liver and excreted in the urine, respectively. The pegvaliase mechanism of action corrects the underlying PAH deficiency that leads to inability to metabolize Phe, increased Phe levels in the body, and consequent neurocognitive, neuropsychiatric, and executive functioning deficits.

A comprehensive review of pegvaliase is contained in the Investigator's Brochure (IB). Investigators must review this document prior to initiating this study.

2.1 Study Rationale

Although it is recommended that adolescents with PKU maintain good metabolic control as they move into adulthood (Vockley 2014), in separate surveys > 60% of adolescents (13 to 17 years of age) in the US have blood Phe concentrations above recommended targets (Jurecki 2017) and 79% of patients 15 to 19 years report blood Phe concentrations above targets (Walter 2002). Adolescents aged 12 to 17 years with PKU report difficulties with maintaining blood Phe control as they transition away from childhood when parental oversight of dietary and blood Phe control decreases (Brown 2016; van Spronsen 2017). In a 2015 National PKU Alliance (NPKUA) survey conducted among over 625 adults and families, approximately 25% of those caregivers indicated that their children's average blood Phe levels were in excess of the ACMG-recommended targeted range (120 to 360 µmol/L), and over half of all respondents (adults and caregivers) reported that it was somewhat to very difficult to manage their PKU (Brown 2016).

Adolescent patients report difficulties adhering to a Phe-restricted diet to maintain blood Phe levels within a safe range (Brown 2016; van Spronsen 2017), and a clinic survey assessing compliance with clinical guidance and dietary restriction showed that 60% of adolescents aged 13 to 17 were non-compliant, with an associated increase of Phe levels to > 360 µmol/L in 50% of patients and Phe levels > 600 µmol/L in 22% of patients. The same survey demonstrated that non-compliance starts as early as ages 5 to 12, and non-adherence to guidelines increases with age.

For adolescent patients at increased risk for loss of metabolic control maintained during childhood, a variety of adverse neuropsychological deficits (including deficits in executive functioning and psychiatric symptoms, such as anxiety, depression, and phobias) develop later in life when there is relaxation of blood Phe control (Huijbregts 2018; Diamond 1997). Adolescent PKU patients with blood Phe levels ≤360 µmol/L have been shown to perform better at neuropsychological and neurocognitive tasks (Huijbregts 2002; Jahja 2014).

The approval of Palynziq addressed a previous unmet medical need, providing adult PKU patients with a treatment that substantially reduced blood Phe concentrations and resulted in the stabilization of Phe above and beyond diet. The reduction and stabilization of blood Phe were associated with improvement in unmanaged symptoms, including neurocognitive symptoms. There is potential for improvement in these outcomes for adolescents as well given the demonstrated efficacy in adults. Therefore, expansion of Palynziq use to include adolescent patients 12 to 17 years with uncontrolled blood Phe concentrations greater than 600 µmol/L on existing management would broaden the patient population who can benefit from Palynziq treatment and address a currently underserved patient population.

The pathogenesis and pathophysiology of PKU, driven by an absence or deficiency in PAH enzyme leading to high Phe concentration, is applicable to both adolescents and adults. The pegvaliase mechanism of action to correct the underlying metabolic deficiency of PAH gene is the same in both adults and adolescents. Adolescent PKU patients are not expected to have specific ongoing developmental changes that could result in a different safety or efficacy outcome with pegvaliase treatment.

2.2 Background

Palynziq received marketing authorization in the US and European Union (EU). The original Biologics License Application (BLA) for Palynziq was approved by the Food and Drug Administration (FDA) at dosages up to 40 mg/day on 24 May 2018, to reduce blood Phe concentrations in adult patients with phenylketonuria (PKU) who have uncontrolled blood Phe concentrations greater than 600 µmol/L on existing management (BLA STN 761079). A supplemental Biologics License Application (sBLA) that included the addition of the maximum maintenance dose of 60 mg/day and long-term safety and efficacy data for adults was approved by the FDA on 06 October 2020. A Risk Evaluation and Mitigation Strategy (REMS) is a required element of the Palynziq approval in the US. Palynziq was approved by the European Commission on 03 May 2019 at dosages up to 60 mg/day to reduce blood Phe concentrations in patients aged 16 years and older who have inadequate blood Phe control (defined as blood Phe levels greater than 600 µmol/L) despite prior management with available treatment options.

BioMarin recognizes that PKU is a serious genetic disease diagnosed at birth that requires strict management of blood Phe levels from birth and continuing through life. Now that a positive benefit to risk profile has been established in adults, BioMarin is proposing this pegvaliase clinical study in adolescent PKU patients (ages 12 to 17 years old (US), inclusive, and 12 to 15 years old (ex-US), inclusive).

2.3 Benefit/Risk Assessment

Regulatory approval of Palynziq in the US and the EU was based on demonstration of efficacy from the Phase 2 and Phase 3 data of the pegvaliase clinical development program. The approval of pegvaliase addressed a previous unmet medical need, providing adult PKU patients with a treatment that substantially reduced blood Phe concentrations and resulted in the stabilization of Phe above and beyond diet. This reduction and stabilization of blood Phe was also associated with improvement in unmanaged symptoms including neurocognitive symptoms. Given the demonstrated efficacy in adults, there is the potential for improvement in these outcomes for adolescents.

The important identified risks for pegvaliase include acute systemic hypersensitivity reaction (equivalent to anaphylaxis for the purpose of this protocol; see Section 8.3.8.1 for how this risk is defined), angioedema, serum sickness, hypophenylalaninemia, persistent arthralgia, and severe injection site reaction (see Section 8.3.8 for event definitions). These events have been effectively treated and managed in the clinical trial and post-marketing settings. Healthcare providers, patients, and observers are trained in the recognition and management of both an acute systemic hypersensitivity reaction or angioedema, including the use of injectable epinephrine (adrenaline) and the need to seek immediate medical assistance. Premedication is required during Induction/Titration Dosing to help mitigate the risk of hypersensitivity reactions, including acute systemic hypersensitivity reactions. While hypophenylalaninemia has not been reported frequently in the post-marketing setting, it was observed during the clinical studies, where it was managed with decreases in pegvaliase dosing and increases in dietary protein intake. Participants will undergo frequent blood Phe monitoring during induction and titration and throughout dosing and will be required to dose reduce with confirmed hypophenylalaninemia (ie, 2 consecutive blood Phe levels < 30 µmol/L).

The important potential risks of treatment with pegvaliase also include potential complications of immune complex formation resulting in end-organ damage, fetal developmental toxicity, and acute systemic hypersensitivity reaction in patients taking other concurrent injectables containing PEG. No evidence of immune complex-mediated end organ damage or any early clinical signs potentially related to immune complex mediated disease have been reported in the clinical trial or commercial setting. Concurrent injectables containing PEG are prohibited during study treatment with the exception of COVID vaccinations (see Section 6.8.1).

The potential risks are justified by the anticipated benefits that may be afforded to adolescent participants. Each participant will have a comprehensive plan that includes frequent

monitoring of adverse events, blood Phe levels, and pregnancy during the study. Safety will be assessed by adverse event reporting and clinical laboratory assessments.

More detailed information about the known and expected benefits and risks and reasonably expected adverse events of pegvaliase may be found in the IB.

2.3.1 Risk Assessment

Table 2.3.1.1: Risks Associated with Study Drug

Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Acute systemic hypersensitivity reaction	In the completed Phase 2 and Phase 3 clinical trials, 16 out of 285 (6%) participants experienced 25 episodes of acute systemic hypersensitivity reactions of any severity. Acute systemic hypersensitivity reactions were managed by administration of adrenaline (44%; 11/25 episodes), corticosteroids (56%; 14/25 episodes), antihistamines (56%; 14/25 episodes), and/or oxygen (8%; 2/25 episodes). Four out of 16 (1%; 4/285) participants experienced a total of 5 episodes of an acute systemic hypersensitivity reaction that were considered severe. The underlying mechanism for acute systemic hypersensitivity reaction observed in clinical trials was non-IgE mediated Type III (immune-complex mediated) hypersensitivity. Acute systemic hypersensitivity reaction was most frequent during the Induction/Titration Phase (5% of participants; 19 episodes over mean treatment duration of 13 months) and decreased in the Maintenance Phase (2% of participants; 6 episodes over a mean treatment duration of 33 months). The risk of an acute systemic hypersensitivity reaction occurring is 6-fold higher in the Induction/Titration Phase compared to the Maintenance Phase.	To mitigate the risk of an acute systemic hypersensitivity reaction, an adult observer is required for a minimum of 1 hour post injection and premedication including H1 and H2 antagonists and an antipyretic is required 2 to 3 hours before each injection until participants reach the Maintenance Phase.[a] Premedication may be considered during the Maintenance Phase at the investigator's discretion. Participants will be provided with 2 epinephrine injectors and instructed to carry both injectors with them at all times. Participants and adult observers will be trained to recognize the signs and symptoms of anaphylaxis and, if these reactions occur, to call for emergency medical support and administer an epinephrine injector. For the duration of the study, each participant will be contacted weekly by study site personnel to monitor for drug administration problems and adverse events (AEs). Risk mitigation measures also include dosing instructions for other hypersensitivity adverse events (Table 6.5.1.1).
Angioedema	In the completed Phase 2 and Phase 3 clinical trials, 21 out of 285 (7%) participants experienced 37 episodes of mild to moderate episodes of angioedema (symptoms include one or more of the following: pharyngeal edema, swollen tongue, lip swelling, mouth swelling,	To mitigate the risk of hypersensitivity reactions, premedication including H1 and H2 antagonists and an antipyretic is required 2 to 3 hours before each injection until participants reach the Maintenance Phase.[a] Premedication may be considered

Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
	eyelid edema and face edema) occurring independent of acute systemic hypersensitivity reaction. Angioedema was most frequent during the Induction/Titration Phase (6% of participants; 27 episodes over mean treatment duration of 13 months) and decreased in the Maintenance Phase (3% of participants; 10 episodes over mean treatment duration of 33 months). The risk of angioedema occurring is 4.5-fold higher in the Induction/Titration Phase compared to the Maintenance Phase.	during the maintenance phase at the investigator's discretion. An adult observer is required for a minimum of 1 hour post injection throughout the study. For the duration of the study, each participant will be contacted weekly by study site personnel to monitor for drug administration problems and AEs. Risk mitigation measures also include dosing instructions for hypersensitivity adverse events (Table 6.5.1.1).
Serum sickness	In the completed Phase 2 and Phase 3 clinical trials, serum sickness was reported in 7 out of 285 (2%) participants. Serum sickness was most frequent during the Induction/Titration Phase (2% of participants; 6 episodes over mean treatment duration of 13 months) and decreased in the Maintenance Phase (0.6% of participants; 1 episode over mean treatment duration of 33 months). The risk of serum sickness occurring is greater than 2-fold higher in the Induction/Titration Phase compared to the Maintenance Phase.	To mitigate the risk of hypersensitivity reactions, premedication including H1 and H2 antagonists and an antipyretic is required 2 to 3 hours before each injection until participants reach the Maintenance Phase of dosing.[a] Premedication may be considered during the Maintenance Phase at the investigator's discretion. An adult observer is required for a minimum of 1 hour post injection throughout the study. For the duration of the study, each participant will be contacted weekly by study site personnel to monitor for drug administration problems and AEs. Risk mitigation measures also include dosing instructions for hypersensitivity adverse events (Table 6.5.1.1).
Persistent arthralgia (> 6 month duration)	In the completed Phase 2 and Phase 3 clinical trials, 245 out of 285 (86%) participants experienced episodes consistent with arthralgia (including back pain, musculoskeletal pain, pain in extremity, and neck pain). Arthralgia occurred as early as the first dose and can occur at any time during treatment. Arthralgia was most frequent during the Induction/Titration Phase (79% of participants; 1269 episodes over mean treatment duration of 13 months) and decreased in the Maintenance Phase (67% of participants; 673 episodes over mean treatment duration of 33 months). The risk of arthralgia occurring is 3.1-fold higher in the Induction/Titration Phase compared to the Maintenance Phase.	To mitigate the risk of hypersensitivity reactions, premedication including H1 and H2 antagonists and an antipyretic is required 2 to 3 hours before each injection until participants reach the Maintenance Phase of dosing.[a] Premedication may be considered during the Maintenance Phase at the investigator's discretion. An adult observer is required for a minimum of 1 hour post injection throughout the study. For the duration of the study, each participant will be contacted weekly by study site personnel to monitor for drug administration problems and AEs.

Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
	Seven (7%) percent of the participants experienced persistent arthralgia (≥ 6 months). The risk of persistent arthralgia may have significant impact on quality of life. Severe arthralgia (severe pain limiting self-care activities of daily living) was experienced in 5% of participants. Dose was not changed for 96% of episodes and all persistent arthralgia episodes resolved without sequelae.	Risk mitigation measures also include dosing instructions for hypersensitivity adverse events (Table 6.5.1.1).
Severe injection site reaction	Injection site reaction (ISR) was reported in 266 out of 285 (93%) participants. The most common ISR (occurring in at least 10% of participants) was reaction, erythema, bruising, pruritus, pain, swelling, rash, induration, and urticaria. Injection site reaction was most frequent during the Induction/Titration Phase (90% of participants; 3908 episodes over mean treatment duration of 13 months) and decreased in the Maintenance Phase (66% of participants; 1226 episodes over mean treatment duration of 33 months). The risk of an ISR occurring is 5.2-fold higher in the Induction/Titration Phase compared to the Maintenance Phase. Three ISRs consistent with granulomatous skin lesions were reported (each reaction occurring in 1 patient): granulomatous dermatitis (occurred 15 months after pegvaliase treatment and lasted 16 days), xanthogranuloma (occurred 12 months after pegvaliase treatment and lasted 21 months), and necrobiosis lipoidica diabeticorum (occurred 9 months after pegvaliase treatment and lasted 9 months). Necrobiosis lipoidica diabeticorum was treated with steroid injections and was complicated by a *Pseudomonas* infection. All of these injection site reactions resolved.	To mitigate the risk of hypersensitivity reactions, premedication including H1 and H2 antagonists and an antipyretic is required 2 to 3 hours before each injection until participants reach the Maintenance Phase of dosing.[a] Premedication may be considered during the Maintenance Phase at the investigator's discretion. An adult observer is required for a minimum of 1 hour post injection throughout the study. For the duration of the study, each participant will be contacted weekly by study site personnel to monitor for drug administration problems and AEs. Risk mitigation measures also include dosing instructions for hypersensitivity adverse events (Table 6.5.1.1). Any participant who experiences a severe (Grade 3) skin reaction will be asked to consult with a dermatologist for diagnosis and, if clinically indicated, a skin biopsy will be recommended. A Skin Reaction eCRF must be completed.
Hypophenylalaninemia	In the completed Phase 2 and Phase 3 clinical trials, 46% of the participants developed hypophenylalaninemia (blood phenylalanine levels below 30 µmol/L on two consecutive measurements). The risk of hypophenylalaninemia occurring is 2.1-fold higher in the maintenance phase	Blood Phe levels will be frequently monitored during induction and titration dosing and regularly monitored during maintenance dosing in both the Primary Treatment Phase and the Extension Phase of the study. Risk mitigation measures, including protocol-required adjustments in

Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
	compared to the Induction/Titration phase. In the completed adult clinical studies, dose reductions were effective in managing hypophenylalaninemia in participants experiencing hypophenylalaninemia despite appropriate levels of protein intake.	the pegvaliase dose (with or without modifications to the dietary intake of Phe) will be implemented to mitigate the potential risk of chronic hypophenylalaninemia levels (< 30 µmol/L) in adolescent participants.
Complications of immune complex formation resulting in end-organ damage	No evidence from clinical trials or post-marketing reports of immune complex-mediated end organ damage or any early clinical signs potentially related to immune complex mediated disease.	Risk mitigation measures include monitoring clinical laboratory tests (chemistry, hematology, urinalysis) and adverse events for immune complex-mediated end-organ damage. These mitigations will also allow early detection of adverse effects of PEG accumulation. For the duration of the study, each participant will be contacted weekly by study site personnel to monitor for AEs.
Fetal development toxicity	There are limited data from the use of pegvaliase in pregnant women. Animal studies have shown maternal reproductive toxicity that was associated with decreased blood phenylalanine concentrations below normal levels. Uncontrolled blood phenylalanine levels (hyperphenylalaninemia) before and during pregnancy are associated with increased risk for miscarriage, major birth defects (including microcephaly and major cardiac malformations), intrauterine fetal growth retardation and future intellectual disability with low IQ. Additional risk to the unborn child due to hypophenylalaninemia is not established.	If sexually active, male or female participants must not plan to become pregnant (self or partner) and must use 2 acceptable methods of contraception while participating in the study beginning at Screening and for 4 weeks after discontinuing study drug. Contraceptive use must be consistent with the guidance provided in Appendix 4, Section 10.4 of this protocol.
Unpredictable immune-mediated response in patients < 16 years	The adult development program was restricted to participants aged > 16 years of age. Review of spontaneous adverse reaction reports in patients < 16 years of age, using Palynziq in the commercial setting has not identified any unpredictable immune responses. The adverse reactions reported in patients < 16 years of age are consistent with the known safety profile of Palynziq as presented in the USPI and SmPC.	To mitigate the risk of hypersensitivity reactions, premedication including H1 and H2 antagonists and an antipyretic is required 2 to 3 hours before each injection until participants reach the Maintenance Phase. [a] Premedication may be considered during the Maintenance Phase at the investigator's discretion. An adult observer is required for a minimum of 1 hour post injection throughout the study. For the duration of the study, each participant will be contacted weekly by study site personnel to

Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
		monitor for drug administration problems and AEs. Risk mitigation measures also include dosing instructions for hypersensitivity adverse events (Table 6.5.1.1).
Acute systemic hypersensitivity reactions in patients taking other concurrent injectables containing PEG	PEGylated proteins have the potential to elicit an immune response. Because antibodies bind to the PEG portion of pegvaliase, there may be potential for binding with other PEGylated therapeutics and increased hypersensitivity to other PEGylated injectables. In a single dose study of pegvaliase in adult participants with PKU, two patients receiving concomitant injections of medroxyprogesterone acetate suspension containing PEG experienced hypersensitivity reactions.	Risk mitigation measures include prohibiting any injectable drugs containing polyethylene glycol (PEG; other than pegvaliase), including medroxyprogesterone injection, within 3 months prior to the start of Screening/Run-in and during study participation with the exception of COVID vaccinations (see Section 6.8.1).

[a] Participants are in the Maintenance Phase when they achieve blood Phe ≤600 µmol/L while they are on a stable dose of pegvaliase. This is distinct from Maintenance Dosing, defined as the period following Induction/Titration Dosing when Maintenance Doses (20, 40, or 60 mg/day) are administered, as outlined in Table 6.5.1.

2.3.2 Benefit Assessment

The overall clinical benefit of pegvaliase was established in patients with PKU based on data from completed Phase 2 and Phase 3 clinical studies. The approval of Palynziq addressed a previous unmet medical need, providing adult PKU patients with a treatment that substantially reduced blood Phe concentrations and resulted in the stabilization of Phe above and beyond diet. This reduction and stabilization was also associated with improvement in unmanaged symptoms including neurocognitive symptoms. Given the demonstrated efficacy in adults, there is the potential for improvement in these outcomes for adolescents.

2.3.3 Overall Benefit/Risk Conclusion

Results from the robust pegvaliase clinical development program demonstrated that pegvaliase treatment up to 60 mg/day demonstrated a positive benefit-risk profile in patients with PKU with uncontrolled blood Phe levels > 600 µmol/L.

Taking into account the measures taken to minimize risk to participants participating in this study, the anticipated benefits that may be afforded to adolescent PKU patients treated with pegvaliase outweigh the potential risks.

3 OBJECTIVES AND ENDPOINTS

The primary, secondary, and exploratory objectives and associated endpoints are shown in Table 3.1.

Table 3.1: Objectives and Endpoints

Objectives	Endpoints
Primary	
To evaluate the efficacy of pegvaliase in adolescent participants with PKU	The primary efficacy endpoint is change in blood Phe concentration from treatment naïve baseline following 72 weeks on study. Change in blood Phe from baseline is compared between participants in the active (pegvaliase) and the control (diet-only) treatment arms (see Section 9.4.2).
To evaluate the safety of pegvaliase in adolescent participants with PKU	• Adverse events (AEs), including serious AEs (SAEs) • Clinical laboratory tests (chemistry, hematology, and urinalysis) results • Vital signs • Physical examination (including height, weight) • Electrocardiogram (ECG) test results • Immunogenicity test results (anti-PEG IgG, anti-PEG IgM, anti-PAL IgG, anti-PAL IgM, TAb, NAb, and anti-pegvaliase IgE [hypersensitivity reaction visits only]).
Secondary	
To characterize total dietary protein intake in adolescent participants with PKU after pegvaliase treatment	Total dietary protein intake must remain stable for change in blood Phe, the primary endpoint, to be accurately assessed. To gauge the stability of total protein intake, the change from baseline at Week 73 in total dietary protein intake (measured in grams/kilogram [g/kg]) (defined as the sum of the protein intake from medical food and intact food) will be measured (see Section 9.4.3).
To characterize the pharmacokinetics (PK) of pegvaliase in adolescent participants with PKU	Trough plasma PK samples will be collected and analyzed as specified in the SoA. PK parameters assessed with intensive PK sampling will include $AUC_{0\text{-}24hr,ss}$, $C_{max,ss}$, $T_{max,ss}$, $t_{1/2}$, $CL_{ss/F}$, and $V_{dz/F}$. For participants randomized to the active (pegvaliase) treatment arm, intensive PK sampling will be performed at Week 73 and for dose escalations to 20, 40, and 60 mg/day. For participants randomized to the control (diet-only) treatment arm, there are no PK assessments in Part 1. In Part 2, intensive PK sampling will be performed at Week 145 and for dose escalations to 20, 40, and 60 mg/day (see Section 8.4).

Objectives	Endpoints
Exploratory Objectives	
To evaluate the effect of pegvaliase treatment on neurocognitive outcomes in adolescent participants with PKU	• The ADHD Rating Scale IV inattention subscale (ADHD RS-IV) rated by the parent/guardian and performed at Baseline (Day 1, pre-dose) and every 12 weeks throughout the study (see Section 8.1.3.1.1). • The Behavior Rating Inventory of Executive Function, Second Edition (BRIEF2) rated by the parent/guardian and performed at Baseline (Day 1, pre-dose) and every 12 weeks throughout the study (see Section 8.1.3.1.2). • Change from baseline (or first administration) in Phenylketonuria Symptom Severity and Impacts Scale (PKU-SSIS) Version 2.0 scores.
To assess changes in PKU symptoms and impacts	• Change from baseline (or first administration) in global severity items (Patient Global Impression of Severity [PGIS], Caregiver Global Impression of Severity [CaGIS], and Clinician Global Impression of Severity [CGIS]). • Change in global change items (Patient Global Impression of Change [PGIC] and Caregiver Global Impression of Change [CaGIC]).
To compare participants' optimal maintenance pegvaliase dose across different age groups within the study	• Time to participants' optimal maintenance dose compared to older age subsets • Number of participants who require the highest (60 mg/day) maintenance dose • Percentage/number of participants in the blood Phe target ranges of < 600 µmol/L and < 360 µmol/L.
Explore the biochemical, molecular, and cellular aspects of PKU	Blood will be collected to evaluate biochemical, molecular, and cellular aspects of PKU and to develop the assays used for these evaluations.
PAH genotyping	Testing will be performed on samples collected Day 1 pre-dose where allowed by local health authorities and the EC/IRB.

4 STUDY DESIGN

4.1 Overall Design

This Phase 3 multi-center study is designed to evaluate the safety and efficacy of pegvaliase administered daily to adolescent participants (ages 12 to 17 years old (US), inclusive, and 12 to 15 years old (ex-US), inclusive) with PKU. Pegvaliase will be subcutaneously injected in a hospital setting for the first 8 weeks and thereafter at the clinic or at home by a trained participant, caregiver, adult observer, or visiting healthcare provider. Pegvaliase will also be injected in a hospital setting for any subsequent dose escalations. The study design is summarized in Figure 1.2.1.

The study will enroll approximately 54 US and ex-US participants (ages 12 to 17 years old (US), inclusive, and 12 to 15 years old (ex-US), inclusive) who will be evaluated using an open-label, 2-arm, randomized control design, with diet-only management of PKU as the control. Participants will be randomized in a 2:1 ratio to the active and control arms, respectively, with 36 participants receiving pegvaliase and 18 participants managing their PKU with diet alone.

The primary efficacy endpoint is the change from baseline in blood Phe following 72 weeks on study (the end of Part 1). Beginning at Week 73, participants in the active treatment arm will continue to receive pegvaliase in the Extension Phase (Part 2) for up to an additional 142 weeks (through Week 215) in order to evaluate the long-term safety and efficacy of pegvaliase in adolescent participants with PKU. Beginning Week 73, participants in the control treatment arm will initiate pegvaliase treatment and, from Week 73 through Week 215, will follow the same schedule for dosing, study visits, and assessments that the active arm participants followed.

Eligible participants are required to have 2 blood Phe concentration measurements > 600 µmol/L during the Screening/Run-in Period (7 to 10 days apart) and an average blood Phe concentration > 600 µmol/L over the past 12 months prior to Screening based on available historical data. Once enrolled, patients will be dosed with pegvaliase according to the I/T/M dosing regimen based on the US package insert (USPI), with the guidance to increase dose depending on individual blood Phe lowering efficacy (per investigator's discretion). Dose should be increased to 40 mg/day if blood Phe is > 360 µmol/L (recommended) after 24 weeks dosing at 20 mg/day, and increased to 60 mg/day if blood Phe is > 360 µmol/L (recommended) after 16 weeks dosing at 40 mg/day. If a participant's blood Phe is > 600 µmol/L after 16 weeks of continuous treatment with pegvaliase at 60 mg/day,

pegvaliase should be permanently discontinued. BioMarin recommends that the target blood Phe levels should be based on individual blood Phe lowering efficacy.

In Part 1 (through the Week 73 visit), randomized participants in both the control (diet-only) and the active (pegvaliase) treatment arms will be required to maintain the same level of dietary protein intake they established during Screening/Run-in. In Part 2, participants in the control (diet-only) arm must continue to maintain this prescribed level of protein intake, whereas participants randomized to the active (pegvaliase) arm whose blood Phe concentration is ≤ 360 µmol/L for a minimum of 4 weeks may adjust dietary protein intake based on individual participant response to pegvaliase and guidance from the investigator.

4.2 Scientific Rationale for Study Design

This Phase 3 multi-center study is designed to evaluate the safety and efficacy of pegvaliase administered daily to adolescent participants with PKU. Approximately 54 US and ex-US participants (ages 12 to 17 years old (US), inclusive, and 12 to 15 years old (ex-US), inclusive) will be evaluated using an open-label, 2-arm, randomized control design, with diet-only management of PKU as the control.

A RCT design, with diet-only management of PKU as the control, will allow for robust data collection and minimize the risk of confounding factors influencing the results. An open-label diet-only control arm is the best comparator for this age group as daily placebo injections would place undue burden on adolescent PKU patients.

Except for participants randomized to pegvaliase in Part 2 whose blood Phe concentration is ≤ 360 µmol/L for a minimum of 4 weeks, participants in both the control (diet-only) and active (pegvaliase) treatment arms are required to maintain a consistent level of protein intake throughout the study (see Section 4.1 and Section 8.1.2). Participants are required to maintain a consistent level of protein intake to ensure that the safety and efficacy endpoints are attributable to pegvaliase rather than to changes in dietary protein intake.

Pegvaliase will be subcutaneously injected in a hospital setting for the first 8 weeks and for all subsequent dose escalations, and afterwards at the clinic or at home by a trained participant, caregiver, adult observer, or visiting healthcare provider. Administration at home makes daily treatment less burdensome for participants, thereby improving treatment compliance.

An adult observer is required for a minimum of 1 hour post injection throughout the study. An adult observer is defined as someone who will be present with the participant during and after pegvaliase administration, is able to recognize the signs and symptoms of an anaphylaxis episode, and is able to call for emergency medical support and administer

epinephrine, if warranted. The requirement for an adult observer was added to ensure that participants self-administering pegvaliase would have help in recognizing and quickly responding to signs and symptoms of anaphylaxis should they emerge.

The primary efficacy endpoint is the change from baseline in blood Phe following 72 weeks on study (the end of Part 1). Blood Phe level is the established clinical marker used to diagnose PKU and assess disease control, and reduction of blood Phe concentration to target levels is the universally acknowledged treatment goal for PKU patients. Published literature support the clinical benefit of lowering blood Phe concentration to ≤ 600 µmol/L for associated improvement in clinical outcomes including cognitive performance, behavior and mood (Palermo 2017; Romani 2017; Burgard 1999; Azen 1996; Smith 1989; Fitzgerald 2000; Costello 1994; Fonnesbeck 2013; Gassio 2003).

In addition, evidence has shown improved clinical benefit with even more aggressive blood Phe lowering targets. In children and adolescents with PKU, studies show that patients with blood Phe levels < 360 µmol/L perform better at neuropsychological and neurocognitive tasks (Huijbregts 2002; Jahja 2014). The findings from these studies demonstrate the need to adequately control blood Phe levels in PKU pediatric patients to below the 600 µmol/L threshold.

4.2.1 Participant Input into Design

BioMarin sought input from the PKU community in designing this study. Parents and a scientist from the National PKU Alliance reviewed portions of the protocol and provided feedback. Among other important input, the community stressed the importance of:

- Setting expectations for study participation, something that is critical to parents during the informed consent process
- Ongoing support from site personnel during the study
- Training for adult observers
- Simplifying, as much as possible, the diet tracking requirements given the time commitment required on the part of families
- Flexibility in scheduling clinic visits and including home and virtual visits where possible

BioMarin has taken this PKU community feedback into account when designing the study:

- Detailed training will be provided for participants, parents, and adult observers, and will continue throughout the study.
- Diet diaries will be in paper format and, consistent with past studies, diet tracking is restricted to the 3 days prior to study visits.

- Scheduled visits will take place at the clinic or, with the approval of the investigator or designee, virtually. For virtual visits the investigator/designee performs assessments while online with the participant, apart from specimen collections and vital signs, which are performed by home healthcare personnel.

4.3 Justification for Dose and Dose Regimen

Pegvaliase is a daily treatment for patients with PKU that will be administered in an Induction/Titration/Maintenance (I/T/M) regimen described in Section 6.5. Previous clinical studies have suggested the primary clearance mechanism of pegvaliase from plasma is via formation of circulating immune complexes leading to complement activation and removal of the drug by phagocytosis, indicating pegvaliase plasma exposure was driven by immune response. Considering that the immune system matures prior to adolescence (Olin 2018; Georgountzou 2017), the immune response to pegvaliase among adolescent participants is expected to be similar to the responses observed in 16 to 57-year-old study participants in the pegvaliase clinical development program.

Following dosing in a hospital setting for the initial doses (first 8 weeks) and apart from subsequent dose escalations to 20, 40, and 60 mg/day, which will also be administered in a hospital setting, pegvaliase will typically be administered at home by the trained participant, though pegvaliase may be also administered at home by a trained caregiver or visiting healthcare provider. In addition to training in drug administration prior to the first in-clinic dose, participants, caregivers, and adult observers will receive extensive training on how to monitor and respond to possible AEs (including acute systemic hypersensitivity reaction [anaphylaxis]) that may be associated with study drug treatment.

Pegvaliase will be administered using an I/T/M dosing regimen similar to the regimen used in Phase 3 studies performed in the US (165-301 and 165-302) and included in the USPI and SmPC (see Table 6.5.1 and Figure 1.2.2). During pegvaliase clinical development, this dosing regimen was found to help mitigate the onset and severity of hypersensitivity reactions while substantially reducing blood Phe concentrations.

The pegvaliase dose levels in this study have been evaluated in 6 multiple-dose Phase 2 and Phase 3 studies. The dose level for induction in 165-306 will be 2.5 mg once weekly and was chosen because this dose with a once weekly dosing frequency administered during the initial 4 weeks of treatment (ie, induction) used in Studies PAL-002, 165-205, and 165-301 has been associated with a lower incidence and severity of hypersensitivity reactions when compared with 5 days per week dosing frequency at a higher starting dose (0.4 mg/kg) studied in Study PAL-004.

4.4 End of Study Definition

A participant is considered to have completed the study if he/she has completed all scheduled visits, including procedures scheduled at the Study Completion/Early Termination (SC/ET) visit, as shown in the SoA (see Section 1.3), or the study is closed by the sponsor. The end of the study is defined as the date of the last visit of the last participant in the study.

5 STUDY POPULATION

Prospective approval of protocol deviations to eligibility criteria, also known as protocol exemptions, is not permitted. Rescreening is allowed for out of range laboratory values.

5.1 Inclusion Criteria

Participants are eligible to be included in the study only if all of the following criteria apply:

Age

1. Is 12 to 17 years old (US), inclusive, or 12 to 15 years (ex-US), inclusive, at the start of the Screening/Run-in Period (Day -28).

Type of Participant and Disease Characteristics

2. Diagnosis of PKU and failure to maintain recommended blood Phe levels on existing management (defined as failed both sapropterin dihydrochloride treatment and Phe-restricted diet) demonstrated by 2 blood Phe concentration measurements > 600 µmol/L during the Screening/Run-in Period (7 to 10 days in between blood Phe assessments) and average blood Phe concentration > 600 µmol/L over the past 12 months (per available data).
3. Willing and able to maintain and adjust dietary and medical protein food intake according to the study protocol under the supervision of a study dietician or adequately trained designee per investigator discretion during study participation.
4. If on medication for ADHD, depression, or other psychiatric disorder, stable dose of medication for ≥ 8 weeks prior to enrollment and willing to maintain stable dose unless a change is medically indicated.
5. An adult (≥ 18 years of age) has been identified who is willing and competent to observe the participant during study drug administration and for a minimum of 1 hour following administration.
6. Able and willing to comply with all study procedures.
7. Generally in good health, as evidenced by physical examination, clinical laboratory evaluations (hematology, chemistry, and urinalysis), and electrocardiogram (ECG) tests performed at Screening.

Sex and Contraceptive/Barrier Requirement

8. Males and females are eligible to participate in this clinical study.
9. Females with childbearing potential must have a negative pregnancy test at Screening/Run-in and be willing to have additional pregnancy tests during the study.
10. If sexually active, male or female participants must not plan to become pregnant (self or partner) and must use 2 acceptable methods of contraception while participating in the study beginning at Screening and for 4 weeks after discontinuing study drug.

Contraceptive use must be consistent with the guidance provided in Appendix 4, Section 10.4 of this protocol. Acceptable methods of contraception may include:

- Primary forms: hormonal (combination hormone-containing pills, patch, vaginal ring, or intrauterine device) or non-hormonal (copper-containing intrauterine device).
- Secondary forms: barrier forms and other forms of birth control and must include spermicide (eg, male condom; female condom is not an acceptable secondary form).

11. Contraceptive use should be consistent with local regulations regarding the methods of contraception for those participating in clinical studies.

Informed Consent

12. Participants must be capable of giving signed informed consent as described in Appendix 1, Section 10.1, which includes compliance with the requirements and restrictions listed in the informed consent form (ICF) and in this protocol. In the case of participants under the age defined by regional law or regulation, parent(s) or guardian(s) must be willing and able to provide written, signed informed consent after the nature of the study has been explained and prior to performance of any research-related procedure. Participants under the age of majority must be willing and able to provide written assent (if required by local regulations or the IRB/IEC) after the nature of the study has been explained and prior to performance of any research-related procedure.

5.2 Exclusion Criteria

Participants are excluded from the study if any of the following criteria apply:

Medical Conditions:

1. Have a history of a severe systemic hypersensitivity reaction or recurrence of a mild to moderate acute systemic hypersensitivity reaction to any of the excipients or another PEGylated medicinal product.
2. Concurrent disease or condition that would interfere with study participation or safety (eg, history or presence of clinically significant cardiovascular, pulmonary, hepatic, renal, hematologic, gastrointestinal, endocrine, immunologic, dermatologic, neurological, oncologic, or psychiatric disease).

Prior/Concomitant Therapies:

3. Taking any of the prohibited medications outlined in Section 6.8.1.
4. Previous treatment with pegvaliase.
5. Use of any medication that is intended to treat PKU, including the use of large neutral amino acids, within 14 days prior to the administration of study drug on Day 1.

6. Use or planned use of any injectable drugs containing polyethylene glycol (PEG; other than pegvaliase), including medroxyprogesterone injection, within 3 months prior to the start of Screening/Run-in and during study participation with the exception of COVID-19 vaccinations (Section 6.8.1).
7. A history of organ transplantation or on chronic immunosuppressive therapy.

Prior/Concurrent Clinical Study Experience

8. Use of any investigational product or investigational medical device within 30 days prior to Screening/Run-in or requirement for any investigational agent prior to completion of all scheduled study assessments.

Diagnostic Assessments

9. A positive test for HIV antibody, hepatitis B surface antigen, or hepatitis C antibody.
10. Alanine aminotransferase (ALT) concentration > 2 × the upper limit of normal (ULN).
11. Creatinine > 1.5 × ULN.

Other Exclusions

12. A history of substance abuse (as defined by the American Psychiatric Association: Diagnostic and Statistical Manual of Mental Disorders [DSM]) in the past 12 months or current alcohol or drug abuse.
13. Pregnant or breastfeeding or planning to become pregnant (self or partner) or breastfeeding at any time during the study.
14. Major surgery planned during the study period.
15. Inability to identify and/or communicate to others that the participant is experiencing symptoms of potential anaphylaxis due to cognitive impairment or other reasons.
16. Unwillingness or inability of participant/caregiver to carry auto-injectable epinephrine with them at all times.
17. Any condition that, in the view of the investigator, places the participant at high risk of poor treatment compliance or terminating early from the study.

5.3 Lifestyle Considerations

5.3.1 Meals and Dietary Restrictions

Details of all PKU-specific dietary therapy (eg, Phe intake, amino-acid supplementation) must be recorded in the source document and electronic case report form (eCRF).

Dietary Instruction

Participants must maintain dietary Phe intake levels that are consistent with their baseline levels for the duration of the study unless clinically indicated, per dietician or adequately

trained designee per investigator/designee discretion to support individual nutritional needs during study participation (refer to Section 8.1.2).

Fasting Requirements

Fasting (water only) is required for at least 2.5 hours prior to any blood Phe measurement.

Any time fasting is required for visit assessments, the fasting assessments should be completed and fasting stopped before clinical outcome assessments are performed. The fast can be broken with a snack or meal as preferred.

5.3.2 Activity Restrictions

Participants should abstain from strenuous exercise for 48 hours before each blood collection for clinical laboratory tests. Participants may participate in light recreational activities (such as watching television or reading) prior to study visits, as appropriate.

5.4 Screen Failures

Screen failures are defined as participants who assent to participate (and whose parents/guardians consent to their participation) in the clinical study but who are not subsequently enrolled. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and the occurrence of any serious adverse event (SAE).

Individuals who do not meet the criteria for participation in the study may be rescreened. Rescreened participants should be assigned the same participant number as for the initial screening. If eligibility criteria are met on retest within the screening window, the participant may be enrolled after confirmation by the medical monitor. Participants who do not meet eligibility criteria within the screening window, will have to perform all Screening Visit procedures and assessments when re-screened.

5.5 Study Stopping Criteria

The independent Data Monitoring Committee (DMC) will be responsible for performing a benefit to risk assessment of pegvaliase in this patient population on a regular (quarterly) and ad hoc basis during the study and for making recommendations to BioMarin with regards to study conduct. The DMC Chair will be informed of all participants meeting the Individual Stopping Criteria Evaluation for Adverse Events During Pegvaliase Treatment (Section 8.3.8.1.1).

6 STUDY TREATMENT

6.1 Study Treatment(s) Administered

Study treatment is defined as any investigational treatment(s), marketed product(s), placebo, or medical device(s) intended to be administered to a study participant according to the study protocol.

The investigational treatment is pegvaliase (recombinant *Anabaena variabilis* phenylalanine ammonia lyase-PEG). For subcutaneous administration, participants are supplied with prefilled syringes in 3 sizes. Sizes are 2.5 mg (0.5 mL of 5 mg/mL protein concentration), 10 mg (0.5 mL of 20 mg/mL protein concentration), and 20 mg (1.0 mL of 20 mg/mL protein concentration).

The excipients in this drug product are tromethamine, tromethamine-hydrochloride, sodium chloride, trans-cinnamic acid, and water for injection.

Study treatment details are provided in Table 6.1.1.

Table 6.1.1: Study Treatment

Treatment Name	pegvaliase
Type	drug
Dose Formulation	prefilled syringe
Unit Dose Strength	Each PFS is filled to deliver at least 0.5 or 1 mL of formulation containing 2.5, 10, or 20 mg of pegvaliase
Dosage Level(s)	2.5 mg, 5 mg, 10 mg, 20 mg, 40 mg, 60 mg
Route of Administration	subcutaneous injection
IMP and NIMP	IMP
Sourcing	provided centrally by the sponsor
Packaging and Labeling	Drug product packaging is identified with lot and identification numbers and provided in a box labeled with the study number and identified to be for investigational use.
Current/Former Name(s) or Alias(es)	pegvaliase, BMN 165, rAvPAL-PEG (PEGylated recombinant Anabaena variabilis phenylalanine ammonia lyase), Palynziq® (trade name)

IMP, investigational medical products; NIMP, non-investigational medical products; PFS, pre-filled syringe.

In addition to pegvaliase, treatment in this study includes a diet in which total protein intake (defined as the sum of protein intake from medical food and intact food) must remain stable and consistent with baseline levels. Specifically, to accurately assess the primary endpoint comparison between the active (pegvaliase) and control (diet-only) treatment arms on the

change from baseline in blood Phe concentration following 72 weeks on study, total protein intake must remain stable and consistent with baseline levels for participants in both treatment arms through Part 1. In Part 2, participants in the control arm must continue to maintain this prescribed level of protein intake, whereas participants randomized to pegvaliase whose blood Phe concentration is ≤ 360 µmol/L for a minimum of 4 weeks may adjust dietary protein intake based on individual participant response to pegvaliase and guidance from the investigator. Details on diet monitoring are provided in Section 8.1.2.

6.1.1 Additional Drug Administration Instructions

Each prefilled syringe is intended for a single subcutaneous injection. The recommended injections sites include the front middle of thighs and the abdomen at least 2 inches (5 centimeters) away from the navel. If a caregiver is giving the injection, the top of the buttocks and the back of the upper arms are also appropriate injections sites. The injection sites for administration of pegvaliase should alternate between doses. Pegvaliase must not be injected into moles, scars, birthmarks, bruises, rashes, or areas where the skin is hard, tender, red, damaged, burned, inflamed, or tattooed. If multiple injections are needed for a single dose, injections should be administered at the same time of day and injection sites should be at least 5 cm away from each other. Doses should not be divided over the course of the day.

A participant (or participant-designated caregiver) must meet all of the following criteria to be eligible to administer the drug:

- Has no known cognitive impairments that may increase the safety risk per investigator assessment
- Has no medical history or current medications that may compromise administration of pegvaliase per investigator assessment
- Has completed all required drug administration training and has demonstrated administration competency per investigator assessment, including how to prepare pegvaliase for administration and safely perform the injection

Refer to Section 6.5 for study drug administration requirements regarding the presence of an adult observer and for premedication requirements.

Following a dose interruption of ≥ 4 doses, the investigator should obtain approval from the medical monitor prior to the participant restarting pegvaliase. Dose regimens may be revised provided the investigator obtains approval from the medical monitor prior to starting an alternative dose regimen.

The suitability for self-administration of pegvaliase will be reassessed by the investigator if a participant experiences any issues with self-administration. In addition, if a participant has an

NCI-CTCAE grade 3 or higher AE during the study, the participant's suitability for self-administration of pegvaliase will be reassessed by the investigator.

Between scheduled clinic visits, the clinic staff will contact the participant weekly to monitor if the participant is experiencing problems with drug administration, for any AEs, and to answer questions throughout the duration of the study. The participant must perform the scheduled assessments and procedures in the clinic as outlined in the SoA (Section 1.3). Training materials and other information specific to the study site personnel will be provided in the study reference materials, which includes drug administration training materials and instruction on the following:

- How to prepare and safely perform the injections of pegvaliase
- How to receive, store, and return both used and unused pegvaliase
- How to safely use and dispose of prefilled syringes used for injections of pegvaliase
- How to use a prefilled syringe every time drug is administered
- How to care for the injection site after an injection of pegvaliase
- How to document each pegvaliase injection
- How to identify an AE and how to report this to the study site
- How and when to use epinephrine
- Whom to contact at the study site in case of an emergency

Participants will be provided with a workbook to document the date and time of pegvaliase injections, injection site, any suspected AEs, and other relevant information. Dosing in response to an AE should be determined by the investigator.

6.2 Preparation/Handling/Storage/Accountability

At the study site, all study drug must be stored at 2-8°C (36-46°F), under the conditions specified in the IB, and in a secure area accessible only to the designated pharmacists and clinical site personnel. All study drug must be stored and inventoried, and the inventories must be carefully and accurately documented according to applicable state, federal, and local regulations; ICH Good Clinical Practice (GCP); and study procedures. Information regarding storage of study drug in participants' homes is provided in the study training materials.

The investigator or designee must confirm appropriate temperature conditions have been maintained during transit for all study treatment received and that any discrepancies are reported and resolved before use of the study treatment.

Only participants enrolled in the study may receive study treatment, and only authorized site staff may supply study treatment. All study treatment must be stored in a secure, environmentally controlled and monitored (manual or automated) area in accordance with the labeled storage conditions, with access limited to the investigator and authorized site staff.

The investigator, institution, qualified pharmacist, or the head of the medical institution (where applicable) is responsible for study treatment accountability, reconciliation, and record maintenance (ie, receipt, reconciliation, and final disposition records).

Participants are provided instructions for returning used and unused study drug prefilled syringes and for proper disposal at each site visit.

Further guidance and information for the final disposition of unused study treatments are provided in the Pharmacy Manual.

6.3 Measures to Minimize Bias: Randomization and Blinding

This is an open-label study; however, the specific intervention to be taken by a participant will be assigned using an IVRS/IWRS. The site will contact the IVRS/IWRS prior to the start of study intervention administration for each participant. The site will record the intervention assignment on the applicable case report form, if required. Potential bias will be reduced by central randomization.

6.4 Study Treatment Compliance

When participants are dosed at the site, they will receive study treatment directly from the investigator or designee, under medical supervision. The date and time of each dose administered in the clinic will be recorded in the source documents and recorded in the eCRF. The dose of study treatment and study participant identification will be confirmed at the time of dosing by a member of the site staff other than the person administering the study treatment.

When participants, caregivers, or home health nurses administer study treatment(s) at home, the date, time, and quantity of each dose of study drug administered to each participant must be recorded by the participant, caregiver, or home health nurse in a workbook provided for the study. The site staff will collect the workbook at scheduled clinic visits and transcribe this information to the appropriate eCRF.

These data will be used to assess compliance. Deviation(s) from the prescribed dosage regimen should be recorded in the eCRF. Treatment start and stop dates, including dates for treatment delays and/or dose reductions, should also be recorded in the eCRF.

6.5 Dose Modification

Participants will receive pegvaliase at a concentration of 5.0 or 20.0 mg/mL. Pegvaliase will be administered subcutaneously at dose levels in the range of 2.5 to 60 mg. The minimum dose of pegvaliase is 2.5 mg/week. The maximum allowable daily dose of pegvaliase is 60 mg/day (for a maximum weekly total dose of 420 mg).

The duration of the Primary Treatment Phase (Part 1) is 72 weeks, during which pegvaliase treatment will be initiated for participants randomized to the active (pegvaliase) treatment arm using an I/T/M dosing regimen. The recommended dosing schedule for participants dosing with pegvaliase is shown in Table 6.5.1. Induction will consist of a 4-week period when participants randomized to the active treatment arm will receive pegvaliase subcutaneously with a PFS at a fixed dose of 2.5 mg/week and during which the dosing regimen will not be modified in response to blood Phe concentration. Once participants complete induction they will titrate up to a dose of 10 mg/day (investigators may, at their discretion, include dosing at a 5 mg/day dose level). The dosing frequency will be increased gradually during titration dosing to daily (7 days/week). During Maintenance Dosing the dose will be increased to 20 mg/day, 40 mg/day, and 60 mg/day depending upon individual blood Phe lowering efficacy (per the investigator's discretion). The dose should be increased to 40 mg/day if blood Phe is > 360 µmol/L (recommended) after 24 weeks dosing at 20 mg/day and increased to 60 mg/day if blood Phe is > 360 µmol/L (recommended) after 16 weeks dosing at 40 mg/day. If a participant's blood Phe is > 600 µmol/L after 16 weeks of continuous treatment with pegvaliase at 60 mg/day, pegvaliase should be permanently discontinued. BioMarin recommends that the target blood Phe levels should be based on individual needs, with a target blood Phe ≤ 600 µmol/L.

Duration between dose titration steps may be increased in response to AEs. Dose level reductions due to AEs or hypophenylalaninemia (confirmed with 2 consecutive blood Phe values < 30 µmol/L) may be made at any time during the study at the discretion of the investigator. Phe levels will be regularly monitored, and adjustments in the pegvaliase dose (with or without adjustments to the dietary intake of Phe) will be required to mitigate the potential risk of chronic hypophenylalaninemia levels (< 30 µmol/L) in adolescent participants.

Following completion of Part 1, participants randomized to the active (pegvaliase) treatment arm will enter the Extension Phase (Part 2), during which participants will continue dosing with pegvaliase at their optimal maintenance dose level for up to an additional 142 weeks in order to evaluate the long-term safety and efficacy of pegvaliase in adolescent participants with PKU.

Following completion of Part 1, participants randomized to the control (diet-only) treatment arm in Part 1 will repeat the Part 1 assessment schedule while initiating pegvaliase dosing from Weeks 73 through 215.

Table 6.5.1: Study Drug Dose Titration Schedule

Part 1 Dosing	Duration [a]	Dose (mg)	Frequency of Administration (Doses Per Week)
Induction	Week 1	2.5	1
	Week 2	2.5	1
	Week 3	2.5	1
	Week 4	2.5	1
Titration	Week 5	2.5	2
	Week 6	10	1
	Week 7	10	2
	Week 8	10	4
	Week 9	10	7
Maintenance	20 mg daily: 24 weeks [b] 40 mg daily: 16 weeks [b,c] 60 mg daily: maximum dose [c]	20	7
		40	7
		60	7

[a] Minimum duration prior to dose escalation. Dose escalation may be delayed depending on participant tolerability. Investigators may, at their discretion, include dosing at a 5 mg/day dose level as part of the titration from 2.5 mg/week up to 10 mg/day.

[b] Prior to initial dose escalation, the minimum amount of time at 20 mg/day is 24 weeks and the minimum amount of time at 40 mg/day is 16 weeks. The dose should be increased to 40 mg/day if blood Phe is > 360 µmol/L (recommend) after 24 weeks dosing at 20 mg/day, and increased to 60 mg/day if blood Phe is > 360 µmol/L (recommend) after 16 weeks dosing at 40 mg/day.

[c] If multiple injections are needed for a single dose, the injections should be administered at the same time of day and injection sites should be at least 5 cm away from each other. Doses should not be divided over the course of the day. If a participant's blood Phe is > 600 µmol/L after 16 weeks of continuous treatment with pegvaliase at 60 mg/day, pegvaliase should be permanently discontinued.

Following enrollment, participants (and an adult caregiver) will be trained on how to administer study drug at home. Pegvaliase dosing will be conducted in a hospital setting for at least the first 8 weeks including titration up to 10 mg once daily, and for all subsequent dose escalations. The participant (and the adult caregiver) must demonstrate competency with injecting study drug in order to be permitted to administer study drug at home. Dosing at home will not be allowed until the principal investigator has assessed the participant's and

adult observer's competency in proper study drug administration and recognition and appropriate management of the potential risks.

Throughout the study, an adult observer must be present during study drug administration and for a minimum of 1 hour following administration. An adult observer is defined as someone who will be present with the participant during and after pegvaliase administration, is able to recognize the signs and symptoms of an anaphylaxis episode, and is able to call for emergency medical support and administer epinephrine, if warranted. Administration of study drug may only be performed if this adult observer is present. Participants and the adult observers who are observing them during study drug administration will be provided with information and extensive training regarding how to recognize a possible allergic reaction, the severity of the reaction, and instructions on what to do if a reaction occurs. Home administration may not performed until the investigator has assessed the adult observer's competency in proper study drug administration and the recognition and appropriate management of potential risks.

Participants will be provided with 2 epinephrine injectors and are required to carry both injectors with them at all times. Participants and adult observers will be trained to recognize the signs and symptoms of anaphylaxis and, if these reactions occur, to call for emergency medical support and administer an epinephrine injector. For the duration of the study, each participant will be contacted weekly by study site personnel to monitor for administration problems and AEs (see Section 8.3.8.1 for details on the management of suspected anaphylaxis in this study).

All participants receiving pegvaliase must be premedicated with an H1 antagonist, and an H2 antagonist, and an antipyretic approximately 2 to 3 hours prior to each dose until they reach the Maintenance Phase, defined as when participants achieve blood Phe ≤600 µmol/L while they are on a stable dose of pegvaliase. Premedication may be considered during the Maintenance Phase at the investigator's discretion, eg, approximately 2 to 3 hours prior to study drug for 1 week upon reintroduction of study drug, upon resolution of an AE, following any dose interruption of ≥ 4 days, and for any dose increases in the Extension Phase. Participants may also be premedicated at any other time in the study per investigator discretion.

This premedication regimen was successfully implemented in the Phase 3 adult clinical program. Participants will be provided with a workbook to document the date and time of study drug injections, the injection site, premedication use, and suspected AEs.

If ≥ 4 consecutive doses are missed for reasons other than safety related issues, the investigator should obtain approval from the medical monitor prior to the participant resuming study drug.

6.5.1 Dosing Modifications in Response to Hypersensitivity Adverse Events

Dosing modifications in response to a hypersensitivity adverse event (HAE) depends on the NCI-CTCAE grade and suspected relationship study drug. Dosing instructions are provided in Table 6.5.1.1 (regardless of previous occurrence). Dosing modifications are per reported HAE, independent of prior reported HAEs.

Table 6.5.1.1: Dosing Instructions in Response to a Hypersensitivity Adverse Event

NCI-CTCAE Grade [a]	Related to Study Drug	Action with Study Drug			Individual Stopping Criteria Evaluation [d]	HRV Assessment [e]
		Maintain [b]	Reduce [c]	Interrupt [c]		
1	Yes or No	X	(X) Optional	(X) Optional		Investigator discretion
2	Yes or No	X	(X) Optional	(X) Optional		Investigator discretion
3	No				X Immediately consult with sponsor medical monitor	Investigator discretion
3 [d]	Yes			X	X Immediately consult with sponsor medical monitor	Yes (if within 24 hours of onset)
4 [d]	Yes or No			X	X Immediately consult with sponsor medical monitor	Yes (if within 24 hours of onset)

AE, adverse event; CTCAE, Common Terminology Criteria for Adverse Events, Version 5.0; HRV, Hypersensitivity Reaction Visit; NCI, National Cancer Institute.

[a] NCI-CTCAE grade determination is performed by the investigator and may be done either via telephone or clinic visit.

[b] The investigator will instruct the participant to maintain the study drug dose at the time of AE onset until improvement to Grade 1 or resolution (per investigator assessment in the clinic or via telephone).

[c] The study drug dose may be reduced or interrupted if necessary per investigator determination.

[d] If a participant has an NCI-CTCAE Grade ≥ 3 hypersensitivity AE that is related to study drug and is suspected to meet Brown's criteria for severe (Grade 3) in the judgment of the investigator and/or the sponsor's medical monitor, the participant will be permanently discontinued from study drug.

[e] If the investigator determines that the NCI-CTCAE Grade ≥ 3 hypersensitivity reaction is related to administration with study drug, the participant will be asked to return to the clinic within 24 hours of event onset for evaluation, including laboratory tests (chemistry, hematology, urinalysis, anti-pegvaliase IgE [sampling should be performed 8 to 24 hours after event onset and before the next dose of study drug], urine albumin/creatinine ratio, CRP, C3, and C4).

Once an HAE (other than an anaphylaxis episode) improves to Grade 1 or resolves, the study drug dose may be increased, maintained, or reduced, at the discretion of the investigator. If reduced, reductions should be from 60 to 40 mg/day, from 40 to 20 mg/day, or from 20 to 10 mg/day, whichever is appropriate.

6.6 Treatment after the End of the Study

There is no treatment following completion of the study.

6.7 Overdose

For this study, any dose of pegvaliase greater than the prescribed dose will be considered an overdose.

BioMarin does not recommend specific treatment for an overdose. In the event of an overdose, the investigator should contact the BioMarin medical monitor immediately.

6.8 Concomitant Therapy

Any medication or vaccine (including over-the-counter or prescription medicines, vitamins, and/or herbal supplements) that the participant is receiving at the time of enrollment or receives during the study must be recorded on the designated eCRF along with:

- Reason for use
- Dates of administration including start and end dates
- Dosage information including dose and frequency

The medical monitor should be contacted if there are any questions regarding concomitant or prior therapy. The investigator may prescribe additional medications, deemed necessary to provide adequate prophylactic or supportive care, during the study, as long as the prescribed medication is not prohibited by the protocol (refer to Section 5.2). In the event of an emergency, any needed medications may be prescribed without prior approval, but the medical monitor must be notified of the use of any contraindicated medications immediately thereafter. Any concomitant medications added or discontinued during the study should be recorded on the eCRF. Medications should, whenever possible, not be recorded in the electronic database with a frequency of PRN.

6.8.1 Excluded Concomitant Medications

The following medications are prohibited starting at the time specified and until the end of the study:

- Any medication that is intended to treat PKU, including the use of large neutral amino acids, within 14 days prior to the administration of study drug on Day 1.

- Any injectable drugs containing polyethylene glycol (PEG; other than pegvaliase), including medroxyprogesterone injection, within 3 months prior to the start of Screening/Run-in and during study participation with the exception of COVID-19 vaccinations. Please contact the medical monitor prior to administering COVID-19 vaccinations.
- Use of any investigational product or investigational medical device within 30 days prior to Screening/Run-in or requirement for any investigational agent prior to completion of all scheduled study assessments.

In addition, if a participant is on medication for ADHD, depression, or other psychiatric disorder, the dose must be stable for ≥ 8 weeks prior to enrollment and must continue to be stable throughout the study unless a change is medically indicated.

6.8.2 Permitted and/or Required Concomitant Medications

All prescription and over-the-counter medication including herbal supplements and medical food taken by a participant for 30 days before screening are recorded on the designated CRF. Psychiatric medications and their dosage should remain consistent throughout the study unless a change is medically indicated. No new psychiatric medication should be initiated during the study unless medically indicated. No psychotherapy should be initiated during the study unless medically indicated. The investigator may prescribe additional medications during the study as long as the prescribed medication is not prohibited by the protocol (see Section 6.8.1). In the event of an emergency, any needed medications may be prescribed without prior approval but the medical monitor must be notified of the use of any contraindicated medication immediately thereafter. Any concomitant medication added or discontinued during the study should be recorded on the appropriate CRF.

All participants receiving pegvaliase will be required to premedicate with an H1 antagonist, and an H2 antagonist, and an antipyretic approximately 2 to 3 hours prior to each dose of study drug until they reach the Maintenance Phase, defined as when participants achieve blood Phe ≤600 µmol/L while they are on a stable dose of pegvaliase. Premedication may be considered during the Maintenance Phase at the investigator's discretion. For further information regarding premedication, refer to Section 6.5.

7 DISCONTINUATION OF STUDY TREATMENT AND PARTICIPANT WITHDRAWAL FROM STUDY

7.1 Discontinuation of Study Treatment

In rare instances it may be necessary for a patient to permanently discontinue study treatment. If a participant's blood Phe is > 600 µmol/L after 16 weeks of continuous treatment with pegvaliase at 60 mg/day, pegvaliase should be permanently discontinued.

If study treatment is permanently discontinued, the participant will be asked to continue to provide consent at the time of treatment discontinuation to remain in the study and undergo the scheduled study assessments off-treatment. Refer to the SoA for data to be collected at the time of discontinuation of study treatment and follow-up, and for any further evaluations that need to be completed.

If the participant declines to provide consent to remain in the study, the study completion visit assessments should be performed as detailed in the SoA (Section 1.3).

7.2 Participant Withdrawal from the Study

A participant may withdraw from the study at any time at his or her own request, or may be withdrawn at any time at the discretion of the investigator for safety, behavioral, compliance, or administrative reasons.

At the time of discontinuing from the study, if possible, an early termination (ET) visit should be conducted, as shown in the SoA (Section 1.3).

The participant will be permanently discontinued both from the study treatment and from the study at that time.

If the participant withdraws consent for disclosure of future information, the sponsor may retain and continue to use any data collected before such a withdrawal of consent.

If a participant withdraws from the study, BioMarin will only use the remaining samples to complete study procedures as described in Section 8 and no new samples will be collected. If a participant no longer wants the remaining samples to be used for future research, he/she shall inform the study investigator or institution accordingly and BioMarin will only use the remaining samples for health authority requests related to the activities in Section 8, not for future research. The investigator must document this in the site study records.

7.3 Lost to Follow-Up

A participant will be considered lost to follow-up if he or she repeatedly fails to return for scheduled visits and cannot be contacted by the site.

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

- The site must attempt to contact the participant to reschedule the missed visit as soon as possible, counsel the participant on the importance of maintaining the assigned visit schedule, and ascertain whether or not the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow up, the investigator or designee must make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record.
- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study.

Discontinuation of specific sites or of the study as a whole are handled as part of Appendix 1, Section 10.1.9.

8 STUDY ASSESSMENTS AND PROCEDURES

An ICF must be signed and dated by the participant's legally authorized representative, the investigator or designee, and a witness (if required) before any study-related procedures are performed.

All screening evaluations must be completed and reviewed to confirm that potential participants meet all eligibility criteria. The investigator will maintain a screening log to record details of all participants screened and to confirm eligibility or record reasons for screening failure, as applicable. Procedures conducted as part of the participant's routine clinical management (eg, blood count) and obtained before signing of the ICF may be utilized for screening or baseline purposes provided the procedures met the protocol-specified criteria and were performed within the time frame defined in the SoA.

Potential participants will enter the Screening/Run-in period, which will last 4 weeks and include 2 assessments of blood Phe concentration 7 to 10 days apart. Diet diaries should be completed for the 3 days preceding each of the blood Phe assessments. Following confirmation that the 2 blood Phe results are > 600 µmol/L and all other eligibility criteria have been met, participants may be enrolled in the study on Day 1. Protocol exemptions for eligibility are not allowed. Following enrollment, participants will be randomized 2:1 to the active (pegvaliase) or control (diet-only) treatment arm of the study.

In the Primary Treatment Phase (Part 1), participants in the active (pegvaliase) and control (diet-only) treatment arms will follow the same visit schedules and perform the same assessments except that the control participants will not be receiving pegvaliase so will not have PK draws or immunogenicity assessments. The timing of Part 1 assessments for active and control participants is provided in Table 1.3.1 and Table 1.3.4, respectively.

The primary efficacy endpoint, the change from baseline blood Phe, is assessed following 72 weeks on study (the end of Part 1). Beginning Week 73, participants in the active treatment arm will continue to receive pegvaliase in the Extension Phase (Part 2) for up to an additional 142 weeks (through Week 215). The timing of Part 2 assessments for active treatment participants is provided in Table 1.3.2 and Table 1.3.3.

Beginning Week 73, participants in the control treatment arm will initiate pegvaliase treatment and, from Week 73 through Week 215, will follow the same schedule for dosing, study visits, and assessments that the active arm participants followed. The timing of Part 2 assessments for control participants is provided in Table 1.3.5 and Table 1.3.6. All the assessments, dietary rules, and other restrictions that applied to active participants in the Primary Treatment Phase (Part 1) apply to control participants in Part 2 until the Week 145

visit when change from baseline blood Phe for these participants will be assessed after 73 weeks of pegvaliase treatment.

The last scheduled visit, which includes study drug administration, is at Week 215 for participants randomized to both the active (pegvaliase) and control (diet-only) treatment arms. Participants should perform the Study Completion (SC) Visit 4 weeks after the last dose of study drug or after completion of study participation, whichever occurs last. For participants who terminate from the study early, the Early Termination (ET) visit should occur within 4 weeks after the last study drug dose or after withdrawal from study participation, whichever occurs last. If study drug is discontinued before study completion, the investigator will ask the participant to remain in the study to continue study visits and assessments, provided the participant's health, safety, and welfare would not be detrimentally affected.

Immediate safety concerns should be discussed with the sponsor (medical monitor) immediately upon occurrence or awareness to determine if the participant should continue or discontinue study treatment.

Adherence to the study design requirements, including those specified in the SoA, is essential and required for study conduct.

8.1 Efficacy Assessments

Planned timepoints for all efficacy assessments are provided in the SoA (refer to Section 1.3).

8.1.1 Blood Phe Level

The primary efficacy endpoint is change in blood Phe concentration from treatment naïve baseline following 72 weeks on study (see Section 9.4.2). Blood samples for blood Phe analysis are to be drawn according to the SoA (see Section 1.3). Monitoring of blood Phe levels is the cornerstone of diagnosis and treatment of PKU patients. Guidelines for the treatment of PKU are based on blood Phe levels (Vockley 2014; van Wegberg 2017), and clinicians and patients use blood Phe concentration to assess and monitor PKU disease progression and control.

Participants must fast for at least 2.5 hours prior to blood Phe assessments to lessen the effect of food intake on blood Phe concentration. Samples for blood Phe concentration will be analyzed by a central laboratory.

8.1.2 Total Dietary Protein Intake, Dietary Monitoring, and Dietary Protocol Restrictions

Protein from medical foods (Phe-free amino acid fortified food sources) and protein from intact foods (any other food sources containing Phe) are collectively referred to as total dietary protein. Although total dietary protein intake is not an efficacy endpoint in this study, it is an important outcome measure that must remain stable for the primary endpoint to be accurately assessed and under the supervision of a dietician or adequately trained designee per the investigator during study participation. Specifically, to accurately assess the primary endpoint comparison between the active (pegvaliase) and control (diet-only) treatment arms on the change from baseline in blood Phe concentration following 72 weeks on study, total protein intake must remain stable for participants in both treatment arms during this period. It is essential that participants maintain stable protein intake to ensure that the measured changes in their blood Phe are attributable to study treatment rather than changes in their dietary protein intake. Total dietary protein is measured in grams/kilogram (g/kg).

A participant's diet is monitored using a 3-day diet diary. The 3-day dietary record (diet diary) is completed by participants, brought to clinic visits for review, and maintained with the study source documents. Participants record all food, beverages, special low-protein foods, and medical foods consumed for 3 consecutive days prior to the clinic visit and data are analyzed by the nutritional software (Metabolic Pro®) for total kcals, protein, Phe, tyrosine, and the percentage of daily recommended intake provided for protein, Phe, tyrosine, vitamins, and minerals.

A dietitian under investigator supervision is required to monitor and manage the participant's diet for the entire duration of the study, though an investigator or designee may also perform this role. Participants will be provided with 3-day diaries in which all dietary protein intake (including medical food and/or intact food) must be recorded for 3 consecutive days immediately prior to each scheduled clinic visit for review with the dietitian. The participant may be asked to repeat the 3-day diary collection if the dietitian or investigator/designee considers the diary has not been completed accurately; in such circumstances, study visits will be rescheduled. All participants will be provided the option of tyrosine supplementation (500 mg, 3 times per day with meals) at the discretion of the investigator.

The change from baseline in total protein intake at Week 73 will be compared as a secondary endpoint. For this endpoint, baseline protein intake is defined as the average of protein intake values derived from diet diaries collected during Screening/Run-in. During Screening/Run-in, 3-day diet diaries are completed 3 times, each time covering the 3 days periods prior to blood Phe assessments (and collected on the day of those assessments). The

3 Screening/Run-in blood Phe assessments are scheduled to occur (1) at Day -25 to Day -23; (2) 7 to 10 days later (ie, in the Day -18 to Day -13 window); and (3) at Day 1 pre-dose. Thus, the 3 day diet assessments will assess diet for (1) the 3 days within the Day -28 to Day -23 window immediately prior to the first blood Phe assessment; (2) the 3 days within the Day -21 to Day -15 window immediately prior to the second blood Phe assessment; and (3) for Days -3 to Day 1.

Participants will be instructed not to change their total dietary protein intake (medical food and/or intact food) during the Screening/Run-in and Primary Treatment Phase (Part 1) of the study. However, modifications to a participant's diet and reductions in study drug dose level must be implemented if blood Phe levels are reduced to < 30 µmol/L and confirmed upon recheck (performed within approximately 2 weeks) (see Section 8.1.2.1).

In Part 2, dietary protein intake may be modified for participants randomized to active (pegvaliase) treatment if blood Phe concentration is ≤ 360 µmol/L for a minimum of 4 weeks. Should that occur, participants may adjust dietary protein intake based on individual participant response to pegvaliase and guidance from the investigator or designee (see Section 8.1.2.2). Participants randomized to the control (diet-only) treatment arm must continue to maintain dietary protein intake consistent with baseline through Week 145 except in the case of blood Phe levels < 30 µmol/L confirmed upon recheck (see Section 8.1.2.1).

Baseline total dietary protein intake will be used as the comparator for subsequent 3-day diet diary entries. Participants will be required to maintain dietary protein intake levels that are consistent with their baseline levels for the entire duration of the study, except as otherwise specified above, with a consistent diet defined as one in which the intact protein changes are < 15% from baseline and the medical food protein changes are < 15% from baseline.

If a participant demonstrates ≥ 15% change from baseline intake of total intact protein and/or medical food protein, the dietitian (or investigator/designee) will counsel the participant to resume their baseline diet and may perform another 3-day diet record and blood Phe concentration assessment in 2 weeks. If the participant demonstrates ≥ 15% increase in intake of protein from intact foods and/or medical food at the next scheduled study visit, a discussion with the medical monitor may be required to discuss further actions related to non-adherence with consistent dietary protein intake throughout the study, at the discretion of the investigator.

If dietary protein intake is determined to be inconsistent with baseline values, the study site dietitian (or investigator/designee) should counsel the participant on how to return to baseline values by modifying intake of protein from intact foods and/or medical food. If diet changes have been implemented, a blood Phe concentration assessment and 3-day diet record should

be collected 2 weeks after implementation, in addition to all regularly scheduled assessments. Any changes must also be documented on the appropriate eCRF.

8.1.2.1 Diet Modification for Blood Phe Levels Less than 30 µmol/L

In addition to protocol required dose reductions (Section 6.5), modifications to participant's diet should be performed if blood Phe levels are < 30 µmol/L and confirmed upon repeat blood Phe measurement (performed within approximately 2 weeks). Participants will receive counseling for modification of intake of intact protein and medical foods based on intake at the time of blood Phe assessment, as follows:

- If it is determined that a participant is consuming < 2x their recommended level of intact protein per Dietary Reference Intake (DRI) guidelines (1.6 g/kg/day), the dietitian may instruct the participant to increase their intact protein by 20 grams/day and decrease their medical food protein by 20 grams/day.
- If it is determined that a participant is consuming ≥ 2x their recommended level of intact protein per DRI guidelines, the dietitian may instruct the participant to increase their intact protein by 10 grams/day and decrease their medical food protein by 10 grams/day.

Further increases in protein intake as per above are allowed if blood Phe levels are < 30 µmol/L in order to achieve 2 x DRI guidelines (1.6 g/kg/day) of intact protein intake. This increased intake will now be the new reference baseline for dietary protein intake for future dietary assessments. Medical food may be discontinued once the dietitian determines that the essential amino acids meet the appropriate levels for age.

8.1.2.2 Dietary Changes for Blood Phe ≤360 µmol/L in Extension Phase (Participants Randomized to Pegvaliase)

Once participants initially randomized to active (pegvaliase) treatment enter the Extension Phase (Part 2) of the study, dietary protein intake may be modified if blood Phe concentration reaches ≤ 360 µmol/L for a minimum of 4 weeks. If diet is modified, it is recommended to increase protein from intact foods in 10 or 20 gram increments depending on intact protein intake, and decrease protein from medical food by a similar amount, 10 or 20 grams unless the participant is already consuming the appropriate protein levels for age (World Health Organization 2007). Medical food may be discontinued once the dietitian or investigator/designee determines that the essential amino acids provided from intact protein foods meet the appropriate levels for age.

From the beginning of the Extension Phase (Part 2), participants initially randomized to active (pegvaliase) treatment who have achieved blood Phe > 30 to ≤360 µmol/L may have their medical food intake reduced or discontinued and intact protein intake increased, in

accordance with Table 8.1.2.2.1. Implementation of dietary adjustments will be performed in consultation with the site dietitian.

Table 8.1.2.2.1: Dietary Adjustment Algorithm in Part 2 for Participants Randomized to Active (Pegvaliase) Treatment

Phe Category [a]	Protein Intake from Intact Food Sources [b]	Action	
		Protein Intake from Intact Food Sources	Protein Intake from Medical Food
> 30 [d] to ≤ 360 µmol/L	> 2 × RDI (> 1.7 g/kg)	Maintain or reduce by 10 g/day [c]	Discontinue [c]
	> 1 × to 2 × RDI (> 0.8 to 1.7 g/kg)	Maintain	Discontinue [c]
	0.5 × to 1 × RDI (0.4 to 0.8 g/kg)	Increase 10 g/day [c]	Decrease 10 g/day [c]
	< 0.5 × RDI (< 0.4 g/kg)	Increase 20 g/day [c]	Decrease 20 g/day [c]

RDI, recommended daily intake.

[a] Based on results of the previous blood Phe assessment; blood Phe reductions may not be observed until the participant is titrated to an effective dose.

[b] RDI (0.83 g/kg) is based on the joint FAO/WHO/UNU Expert Consultation on Protein and Amino Acid Requirements in Human Nutrition (World Health Organization 2007).

[c] The resulting adjusted dietary protein intake will become the new reference baseline for dietary protein intake for future dietary assessments.

[d] If a participant experiences blood Phe levels < 30 µmol/L on two consecutive blood tests, refer to the action to take in the event of blood Phe ≤ 30 µmol/L (Section 8.1.2.1).

8.1.3 Exploratory Assessments

8.1.3.1 Neurocognitive Assessments

The ADHD RS-IV and BRIEF2 instruments will be used to evaluate the effect of pegvaliase treatment on neurocognitive outcomes in adolescent participants with PKU. The same parent/guardian must complete both the ADHD RS-IV and the BRIEF2 at all scheduled time points.

8.1.3.1.1 The ADHD Rating Scale IV (ADHD RS-IV) Inattention Subscale

The ADHD RS-IV (American Psychiatric Association 2013; DuPaul 1998) will be used to assess inattention and hyperactivity symptoms among the adolescents enrolled in this study. The ADHD RS-IV is derived directly from diagnostic criteria delineated in the DSM, is well validated, and has been extensively used in a broad array of disease classes, including PKU (Gassio 2010; Wyrwich 2016). The ADHD RS-IV was the secondary endpoint in the

BioMarin Phase 3 pegvaliase study (165-302) in which improvement in inattention symptoms was associated with reduction in mean blood Phe levels (Thomas 2018).

The ADHD RS-IV instrument is completed independently by the parent/guardian. The same parent/guardian must complete the scale at each scheduled time point. The instrument consists of 2 nine-item scales that evaluate symptom severity for inattention and hyperactivity/impulsivity, respectively, during the previous month. Each scale is scored separately. Each item is rated on a 4-point severity scale (0, none; 1, mild; 2, moderate; 3, severe); scores of 2 or 3 on an individual item indicate a symptom. The PKU literature suggests that inattentive symptoms are more prevalent than hyperactivity and impulsivity symptoms among individuals with PKU; thus, the inattentive sub-scale is considered the most relevant and will be included in analyses (Bilder 2016).

8.1.3.1.2 Behavior Rating Inventory of Executive Function, Second Edition (BRIEF2)

The BRIEF2 is a 63-item measure designed to assess executive function skills in children 5 to 18 years of age (Gioia 2002). The BRIEF2 was developed to address the limitations of available assessments in examining real-word expressions of behaviors related to executive function. The BRIEF2 is intended to provide a standard method of asking multiple raters about executive function in daily life in a manner that is not specific to any particular disease. Because it is non-disorder-specific, BRIEF2 assesses executive function behaviors in children and adolescents experiencing a wide range of difficulties, such as those related to learning, attention, brain injuries, development disorders, and various psychiatric conditions and medical issues. The BRIEF2 is most often used to assess attention deficit hyperactivity disorder.

The BRIEF2 instrument (completed by the parent/guardian) contains 63 items in 8 non-overlapping clinical scales and 2 validity scales. The same parent/guardian must complete the BRIEF2 at each scheduled time point. These scales form 2 indexes: (1) Behavior Regulation (3 scales) and (2) Metacognition (5 scales), as well as a Global Executive Composite score which takes into account all of the clinical scales and represents the adolescent's overall executive function.

8.1.3.1.3 PKU Symptom Severity and Impacts Scale (PKU-SSIS) v2.0 – Adolescent Version

The PKU Symptom Severity and Impacts Scale (PKU-SSIS) was designed to evaluate neuropsychological symptoms and impacts in early-treated patients with PKU. The PKU-SSIS is comprised of 22 items that constitute 3 symptoms domains: 1) Emotional, Mood and Psychological; 2) (Neuro)cognitive, Executive, and Intellectual Function; 3) Physical Health.

It also has 4 impact domains: 1) Social Relations; 2) Level of Independence; 3) General Wellbeing; and 4) Self-care (Quinn 2022). PKU-SSIS Version 2.0 is developed specifically for adolescents 12 to 17 years old. The participant completes the PKU-SSIS v2.0 independently. Each item is scored on a 5-point Likert scale which asks participants to report how frequently they have experienced symptoms over the last 7 days between "Never," "Rarely," "Occasionally," "Frequently," and "Very Frequently." For each point of the Likert scale the following numerical value is given: Never=0, Rarely=1, Occasionally=2, Frequently=3, and Very Frequently=4. Two items on the scale ask the participants to rate their satisfaction with social interactions and leisure time over the last 7 days; these items are scored on a 5-point Likert scale ranging from "Very Dissatisfied" to "Very Satisfied." For each point of the Likert scale the following numerical value is given: Very Dissatisfied=0, Dissatisfied=1, Neutral=2, Satisfied=3, and Very Satisfied=4.

Scores to items 14, 17, 21, and 22 are reversed so that lower scores represent less symptom severity. Each domain score is computed by summing all the item scores within each domain and converting to a percentage score from the highest possible score from that domain. The instrument total score is compiled by summing the domain scores and converting to a percentage score (ie, Total score = (summed score/88)*100). Participants will complete the PKU-SSIS as indicated in the SoA.

8.1.3.2 Global Impression Scales

8.1.3.2.1 Caregiver Global Impression of Severity (CaGIS) and Caregiver Global Impression of Change (CaGIC)

The Caregiver Global Impression of Severity (CaGIS) and Change (CaGIC) items are standard, single items that have been widely used across a broad range of disease areas and are typically included in clinical trials as anchor measures to help establish meaningful change in other COAs. The CaGIS is used to rate the overall disease severity, as reported by the parent/guardian, and includes a 5-point Likert scale from "None/Not at all impacted" to "Very severe/Very severe impact." The CaGIC is a single global item used to assess if there has been an improvement or change in clinical status; the item includes a 5-point Likert scale from "Much better" to "Much worse." Both global and domain specific items will be created for the CaGIS and CaGIC. Parents/guardians will complete the CaGIS and CaGIC at baseline (CaGIS only) and as indicated in the SoA.

8.1.3.2.2 Patient Global Impression of Severity (PGIS) and Patient Global Impression of Change (PGIC)

The Patient Global Impression of Severity (PGIS) and Change (PGIC) items are standard, single items that have been widely used across a broad range of disease areas and are typically included in clinical trials as anchor measures to help establish meaningful change in other COAs. The PGIS is used to rate the overall disease severity, as reported by the participant, and includes a 5-point Likert scale from "None/Not at all impacted" to "Very severe/Very severe impact." The PGIC is a single global item used to assess if there has been an improvement or change in clinical status; the item includes a 5-point Likert scale from "Much better" to "Much worse." Both global and domain specific items will be created for the PGIS and PGIC. Participants will complete the PGIS and PGIC at baseline (PGIS only) and as indicated in the SoA.

8.1.3.2.3 Clinician Global Impression of Severity (CGIS)

The Clinician Global Impression of Severity (CGIS) is a standard, single global item that has been widely used across a broad range of disease areas and are typically included in clinical trials as anchor measures to help establish meaningful change in other COAs. The CGIS is used to rate the overall disease severity, as reported by the investigator or designee clinician, and includes a 5-point Likert scale from "None" to "Very severe." An overall severity item will be created for the CGIS. The investigator or designee clinician will complete the CGIS at baseline and as indicated in the SoA.

8.2 Safety Assessments

Planned time points for all safety assessments are provided in the SoA.

8.2.1 Physical Examination

Physical examinations (full or brief) should be performed at the timepoints indicated in the SoA:

- A full physical examination will include, at a minimum, general appearance (head, eyes, ears, nose, and throat) and an assessment of cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, musculoskeletal, and neurologic systems.
- A brief physical examination may be performed at the discretion of the investigator based on the participant's clinical condition and may include, but is not limited to, general appearance and an assessment of cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, musculoskeletal, and neurologic systems.

Investigators should pay special attention to clinical signs related to previous serious illnesses.

Height and weight will also be measured and recorded at the timepoints indicated in the SoA (Section 1.3).

8.2.2 Vital Signs

Vital signs include respiration rate, temperature, and single measurements of heart rate and seated diastolic and diastolic blood pressure. Any clinically significant change in vital signs will be recorded as an AE. Blood pressure and pulse measurements should be preceded by at least 5 minutes of rest for the participant in a quiet setting without distractions (eg, television, cell phones). Blood pressure and pulse measurements will be assessed with a completely automated device. Manual techniques will be used only if an automated device is not available.

Results will be recorded on the eCRF. Any abnormal vital sign assessments should be repeated once, and repeat value should be recorded in the eCRF.

8.2.3 Electrocardiograms

Electrocardiograms (ECGs) will be performed at Screening, Baseline, and SC/ET, as shown in the SoA (Section 1.3). Single 12-lead ECG will be obtained using an ECG machine that automatically calculates the heart rate and measures PR, QRS, QT, and QTc intervals.

8.2.4 Clinical Safety Laboratory Assessments

See Appendix 2, Section 10.2 for the table of clinical laboratory tests to be performed and refer to the SoA (Section 1.3) for the timing and frequency.

The investigator must review the laboratory report and document this review. The laboratory reports must be filed with the source documents. If known, the underlying diagnosis associated with abnormal clinical laboratory test results that are considered clinically significant by the investigator should be recorded on the AE eCRF. Clinically significant abnormal laboratory findings are those which are not associated with the underlying disease, unless judged by the investigator to be more severe than expected for the participant's condition.

All abnormal laboratory tests with values considered clinically significant per the investigator during participation in the study and 30 days after the last dose of study treatment should be repeated until the values return to normal or baseline or are no longer considered clinically significant by the investigator.

In the event of elevated urinary protein on a test result, a repeat urinalysis should be performed. This repeat urine sample must be performed in the morning at the first or second morning void to allow for accurate test results and may be performed by a home healthcare nurse. Participants with a confirmed urine/albumin creatinine ratio of ≥ 100 mg/g should be

referred to a nephrologist for consultation if results were within normal range at Baseline. Participants who had elevated results at Baseline followed by a confirmed subsequent increase of ≥ 100 mg/g from Baseline should also be referred to a nephrologist for consultation.

If clinically significant values do not return to normal/baseline within a period of time judged reasonable by the investigator, the etiology should be identified and the medical monitor notified.

All protocol-required laboratory assessments, as defined in Appendix 2, Section 10.2, must be conducted in accordance with the laboratory manual and the SoA.

If laboratory values from non-protocol specified laboratory assessments performed at the institution's local laboratory require a change in participant management or are considered clinically significant by the investigator (eg, SAE or AE or dose modification), then the results must be recorded in the eCRF.

8.2.5 Pregnancy Testing

Based on findings in studies of pregnant animals without PKU treated with pegvaliase-pqpz, pegvaliase may cause fetal harm when administered to a pregnant woman. Because the risks of taking study drug during pregnancy and breastfeeding in humans are unknown, participants must not take study drug if they are trying to conceive, are pregnant, or are breastfeeding. From Day –28 until the end of the study, sexually active participants must use 2 acceptable methods of contraception as outlined in the inclusion criteria section of this protocol (Section 5.1). Participants who are confirmed to be pregnant by a serum pregnancy test and are temporarily off study drug are not required to perform the scheduled urine pregnancy tests.

In addition to contraception requirements, females of child-bearing potential are required to have urine pregnancy tests performed at the timepoints specified in the SoA (Section 1.3). Urine pregnancy test should be performed by the local laboratory. A serum pregnancy test will be performed by the central laboratory in the event of a positive or equivocal urine pregnancy test result.

Refer to Section 8.3.5 for details on the reporting procedures in the event of pregnancy.

8.3 Adverse Events and Serious Adverse Events

The definitions of an AE or SAE can be found in Appendix 3, Section 10.3.

AEs will be reported by the participant (or, when appropriate, by a caregiver, surrogate, or the participant's legally authorized representative).

The investigator and any medically qualified designees are responsible for identifying, documenting, and recording events that meet the definition of an AE or SAE and remain responsible for following up AEs that are serious, considered related to the study treatment or study procedures, or that caused the participant to discontinue the study treatment (refer to Section 7).

8.3.1 Time Period and Frequency for Collecting AE and SAE Information

Control (diet-only) treatment arm participant reporting:

- After informed consent to Day 1, only SAEs related to protocol mandated procedures will be collected.
- All AEs, SAEs, AESI, pregnancy and device related events will be collected from Day 1 through 4 weeks after last dose of study treatment or the study completion visit, whichever is longer.
- Non serious events that occur after informed consent but prior to Day 1 will be recorded on the Medical History/Concurrent Medical Conditions eCRF, not the AE eCRF.

Active (pegvaliase) treatment arm participant reporting:

- After informed consent through the first dose of study treatment, only SAEs related to protocol mandated procedures will be collected.
- All AEs, SAEs, AESI, pregnancy, and device-related events will be collected after the first dose of study treatment through 4 weeks after the last dose of study treatment or the study completion visit, whichever is longer. Non serious events that begin after informed consent but before study treatment will be recorded on the Medical History/Concurrent Medical Conditions eCRF, not the AE eCRF.

All SAEs and AESIs must be recorded and reported to the sponsor or designee immediately; under no circumstances should this exceed 24 hours after the site becomes aware of the event, as indicated in Appendix 3, Section 10.3. The investigator must submit any updated SAE data to the sponsor within 24 hours of it being available.

Investigators are not obligated to actively seek AE or SAE information after conclusion of study participation. However, if the investigator learns of any SAE, including a death, at any time after a participant has been discharged from the study and he/she considers the event to be reasonably likely to be related to the study treatment or study participation, the investigator must promptly notify the sponsor.

8.3.2 Method of Identifying AEs and SAEs

The method of recording, evaluating, and assessing causality of AEs and SAEs and the procedures for completing and transmitting SAE reports are provided in Appendix 3, Section 10.3.

Care will be taken not to introduce bias when identifying AEs and/or SAEs. Open-ended and non-leading verbal questioning of the participant is the preferred method to inquire about AE occurrences.

8.3.3 Follow-up of AEs and SAEs

After the initial AE/SAE report, the investigator is required to proactively follow each participant at subsequent visits/contacts. All SAEs and AESIs (as defined in Section 8.3.8) will be followed until resolution, stabilization, the event is otherwise explained (ie, not related to study drug and/or study participation), or the participant is lost to follow-up (as defined in Section 7.3). Further information on follow-up procedures is provided in Appendix 3, Section 10.3.

8.3.4 Regulatory Reporting Requirements for SAEs

Prompt notification by the investigator to the sponsor of a SAE is essential so that legal obligations and ethical responsibilities towards the safety of participants and the safety of a study treatment under clinical investigation can be met.

The sponsor has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a study treatment under clinical investigation. The sponsor will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority, Institutional Review Boards (IRB)/Independent Ethics Committees (IEC), and investigators.

Expedited safety reports must be prepared for suspected unexpected serious adverse reactions according to global regulatory requirements and sponsor policy and forwarded to investigators.

An investigator who receives an expedited safety report or other specific safety information from the sponsor will review and then file it along with the IB and will notify the IRB/IEC as appropriate.

8.3.5 Pregnancy

The importance of pregnancy prevention for study participants and their partners will be communicated to all study participants with regular reminders during the study.

Any female participant who becomes pregnant while participating in the study will temporarily discontinue study treatment.

Details of all pregnancies in female participants and, if indicated, female partners of male participants, will be collected after the start of study treatment and until 4 weeks after the last dose of study treatment.

While pregnancy itself is not considered to be an AE or an SAE, any pregnancy complication or elective termination of a pregnancy for medical reasons will be reported as an AE or SAE.

Abnormal pregnancy outcomes (eg, spontaneous abortion, fetal death, stillbirth, congenital anomalies, ectopic pregnancy), as well as any hospitalizations associated with pregnancy complications, unplanned caesarean section, and delivery complications should always be classified as an SAE (as the sponsor considers these to be medically significant), recorded on the eCRF, and expeditiously reported to the sponsor as an SAE following the procedures outlined in Appendix 3, Section 10.3.4.

The participant (or pregnant female partner) will be followed to determine the outcome of the pregnancy. The investigator will collect follow-up information on the participant (or pregnant female partner) and the neonate, and the information will be forwarded to the sponsor.

Any post-study pregnancy-related SAE considered reasonably related to the study treatment by the investigator should be reported to the sponsor as described in Section 8.3.4. While the investigator is not obligated to actively seek this information in former study participants (or their pregnant female partners), he or she may learn of an SAE through spontaneous reporting.

Pregnancy reporting requirements to BioMarin are shown in Table 8.3.5.1.

Table 8.3.5.1: Pregnancy Reporting to BioMarin

Pregnancy Reporting to BioMarin
• Pregnancy and Pregnancy Outcome information will be entered into the electronic data collection system. • Pregnancy and Pregnancy Outcome information will be reported to BPV via the study specific Pregnancy Report and Pregnancy Outcome paper report forms. The site will complete the forms and submit via facsimile or email without 24 hours of awareness. • Abortion, Stillbirth, Fetal Congenital Anomaly and any other event meeting seriousness criteria must be reported as an SAE via the study electronic data collection system.

Male Participants with Partners Who Become Pregnant

The investigator will attempt to collect pregnancy information on any male participant's female partner who becomes pregnant while the male participant is in this study. This applies only to male participants who receive pegvaliase.

After obtaining the necessary signed informed consent from the pregnant female partner directly, the investigator will record pregnancy information on the appropriate form and submit it to the sponsor within 24 hours of learning of the partner's pregnancy. The female partner will also be followed to determine the outcome of the pregnancy. Information on the status of the mother and child will be forwarded to the sponsor. Generally, the follow-up will be no longer than 6 to 8 weeks following the estimated delivery date. Any termination of the pregnancy will be reported regardless of fetal status (presence or absence of anomalies) or indication for the procedure.

8.3.6 Cardiovascular and Death Events

All deaths that occur during the AE reporting period, regardless of attribution, will be recorded on the AE eCRF and expeditiously reported to the Sponsor as an SAE.

When recording a death, the event or condition that caused or contributed to the fatal outcome should be recorded as the single medical concept on the eCRF. If the cause of death is unknown and cannot be ascertained at the time of reporting, record "Unexplained Death" or "Death of Unknown Cause" on the eCRF.

8.3.7 Disease-Related Events and/or Disease-Related Outcomes Not Qualifying as AEs or SAEs

The following disease-related event (DRE) is common in participants with PKU and can be serious:

- Hyperphenylalaninemia (Medical Dictionary for Regulatory Activities terminology [MedDRA] term: amino acid increased)

Because this event is typically associated with the disease under study, it will not be reported according to the standard process for expedited reporting of SAEs even though the event may meet the definition of a SAE. The elevated blood Phe levels should be recorded in the central laboratory database as per standard practice. These DREs will be monitored on a routine basis.

If either of the following conditions applies, then the event must be recorded and reported as an SAE (instead of a DRE):

- The event is, in the investigator's opinion, of greater intensity, frequency, or duration than expected for the individual participant; OR
- The investigator considers that there is a reasonable possibility that the event was related to study treatment.

8.3.8 Adverse Events of Special Interest

The following adverse events of special interest (AESI) must be reported to BPV within 24 hours of site awareness, irrespective of seriousness, severity, or causality to facilitate real-time monitoring and assessment. All AESI must be reported using the SAE form regardless of seriousness:

- Acute systemic hypersensitivity reaction, anaphylactic, or anaphylactoid reactions (refer to Appendix 5, Section 10.5)
- Angioedema
- Serum sickness
- Severe injection site reaction defined as an injection site reaction that is considered CTCAE Grade ≥ 3
- Hypophenylalaninemia events (defined as blood Phe < 30 µmol/L on 2 consecutive measurements)
- Arthralgia that is either persistent (AE duration > 6 months), severe (CTCAE Grade ≥ 3), or that requires treatment with a steroid (excluding topical)

8.3.8.1 Anaphylaxis (Acute Systemic Hypersensitivity Reaction)

All potential anaphylactic events, however reported, will be reviewed by BioMarin. When an event occurs which an investigator believes is possibly anaphylaxis, in addition to reporting the event to BPV within 24 hours of site awareness, it should be reported to the sponsor's medical monitor within 24 hours of site awareness.

In this protocol the term anaphylaxis is used to denote the important identified risk: acute systemic hypersensitivity reaction (see Section 2.3.1). An acute systemic hypersensitivity reaction (referred to as anaphylaxis in this protocol) is defined as an episode meeting National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network (NIAID/FAAN) criteria according to an internal BioMarin assessment performed by the Internal Data Safety Review Board. The Internal Safety Data Review Board, consisting of a representative from BioMarin clinical sciences, safety, and biostatistics departments, will review all cases of potential anaphylaxis to determine which of these events meet the criteria for an acute systemic hypersensitivity reaction as summarized in Appendix 5, Section 10.5.2.

In the clinical study report, analyses of anaphylaxis will include both the definition used in this protocol of an acute systemic hypersensitivity reaction and the FDA's definition of anaphylaxis, developed by the Division of Pulmonary, Allergy, and Rheumatology Products (DPARP) and used by BioMarin in the Palynziq US prescribing information (see the SAP for further details).

8.3.8.1.1 Individual Stopping Criteria Evaluation for Adverse Events During Pegvaliase Treatment

A participant who has an anaphylaxis episode that is suspected to meet Brown's criteria for severe (Grade 3) and that is, in the judgment of the investigator and/or the sponsor's medical monitor, related to study drug, will be permanently discontinued from study drug. Clinically severe hypersensitivity (Grade 3) per Brown's criteria is defined as significant hypoxia, hypotension or neurologic compromise that is life-threatening or required treatment to prevent a life-threatening event (Brown 2004):

- Cyanosis or $SpO_2 \leq 92\%$
- Hypotension with SBP < 90 mmHg
- Neurologic alteration: confusion, loss of consciousness, collapse, incontinence

In addition, a participant with a recurrent anaphylaxis episode of any severity that is, in the judgment of the investigator and/or the sponsor's medical monitor, related to study drug will be permanently discontinued from treatment.

8.3.8.1.2 Response to Anaphylaxis Episodes

If a participant experiences any symptoms suggestive of an anaphylaxis episode (as taught to the participant and adult observer in the initial and ongoing training on this risk), they will be instructed to inject epinephrine, to seek immediate medical attention, *and* to inform the investigator.

If the investigator suspects that an AE is anaphylaxis as defined by NIAID/FAAN, the participant will be assessed in the clinic and the sponsor's medical monitor must be immediately notified. Laboratory assessments for suspected anaphylaxis events described in the SoA (Section 1.3) under Hypersensitivity Reaction Visit (HRV) and in Table 6.5.1.1 should be performed prior to the next administration of study drug (if applicable) and should include anti-pegvaliase IgE (for optimal results, sampling should be performed 8 to 24 hours after event onset). If the investigator determines it is safe for the participant to resume dosing with study drug following resolution of anaphylaxis at any time during the study (excepting episodes requiring participant discontinuation, as outlined above), the following steps are required:

- At least the first dose administered after resolution of anaphylaxis is to be given at the clinic with equipment for emergency resuscitation (including epinephrine) within easy access.
- The participant must be premedicated with an H1 antagonist, and an H2 antagonist, and an antipyretic (eg, acetaminophen) approximately 2 to 3 hours prior to each dose of study drug for at least 1 week upon resumption of dosing, regardless of the duration of dose interruption.
- As with all doses of pegvaliase, an adult observer must observe the participant during study drug administration and for a minimum of 1 hour following study drug administration.

8.3.8.2 Angioedema

Angioedema is an eruption similar to urticaria but with larger, edematous wheals involving dermal, subcutaneous or submucosal tissues. It is sometimes associated with severe respiratory distress due to edema of the upper airways. Angioedema may present as a symptom (eg, lip, eye, tongue, throat, or facial swelling) of an acute systemic hypersensitivity reaction or anaphylaxis but may also present in isolation.

8.3.8.3 Serum Sickness

Serum sickness is a Type III immune hypersensitivity reaction that occurs when the immune system develops antibodies to a foreign protein. The antibody and foreign protein combine to form an immune complex that can settle in areas of the body such as the joints or the kidney. Typical presentations include signs and symptoms commonly associated with hypersensitivity or infections: rash, itching, fever and arthralgia (MedDRA terms: serum sickness, serum sickness-like reaction and Type III immune-complex mediated reaction).

8.3.8.4 Severe Injection Site Reaction

A severe injection site reaction will be defined as any injection site reaction adverse event that is reported as CTCAE Grade 3 or above in severity and that codes to the MedDRA high level term (HLT) of Injection site reaction.

8.3.8.5 Hypophenylalaninemia

Blood Phe levels will be regularly monitored during the study. Hypophenylalaninemia is defined in this study as 2 consecutive blood Phe levels < 30 µmol/L. Hypophenylalaninemia will be managed in this study as described in Section 6.5.

8.3.8.6 Arthralgia

Arthralgia will be identified using the MedDRA PT of arthralgia and will include any synonyms of arthralgia such as musculoskeletal pain, pain in the extremity, or neck pain.

Any event that meets these criteria and has a duration of > 6 months will be considered a persistent arthralgia. An arthralgia event that is reported as CTCAE Grade 3 or above in severity will be considered a severe event. Arthralgia events that require treatment with a steroid medication will also be considered an AESI.

8.3.9 Medical Device Incidents (Including Malfunctions)

Medical devices (prefilled syringes) are being provided for use in this study. In order to fulfill regulatory reporting obligations, the investigators are responsible for the detection and documentation of events meeting the definitions of device deficiency or malfunction that occur during the study with such devices.

The definition of a Medical Device deficiency can be found in Appendix 6, Section 10.6.

NOTE: Incidents fulfilling the definition of an AE/SAE will also follow the processes outlined in Section 8.3.3 and Appendix 3, Section 10.3.

8.3.9.1 Time Period for Detecting Medical Device Deficiencies

Medical device incidents or malfunctions of the device that result in an incident will be detected, documented, and reported during all periods of the study in which the medical device is used.

If the investigator learns of any device deficiency at any time after a participant has been discharged from the study, and such incident is considered reasonably related to a medical device provided for the study, the investigator will promptly notify the sponsor.

The method of documenting Medical Device deficiencies is provided in Appendix 6, Section 10.6.

8.3.9.2 Follow-up of Medical Device Deficiencies

Follow-up applies to all participants, including those who discontinue study treatment.

The investigator is responsible for ensuring that follow-up includes any supplemental investigations as indicated to elucidate the nature and/or causality of the deficiency.

New or updated information will be recorded on the originally completed form with all changes signed and dated by the investigator.

8.3.9.3 Prompt Reporting of Device Deficiencies to Sponsor

Device deficiencies must be reported to the sponsor within 24 hours after the investigator determines that the event meets the protocol definition of a medical device deficiency.

The investigator should complete the Device Malfunction eCRF in electronic data capture (EDC). The investigator/designee must complete a Product Complaint form and submit it to the sponsor via fax or email as outlined in the Pharmacy Manual. The sponsor will be the contact for the receipt of device deficiency reports.

8.3.9.4 Regulatory Reporting Requirements for Device Deficiencies

The investigator must promptly report all device deficiencies occurring with any medical device provided for use in the study so the sponsor can fulfill their legal responsibility to notify appropriate regulatory authorities and other entities about certain safety information relating to medical devices being used in clinical studies.

The investigator, or responsible person according to local requirements (eg, the head of the medical institution), will comply with the applicable local regulatory requirements relating to the reporting of device deficiencies to the IRB/IEC.

8.4 Pharmacokinetics

Blood samples will be collected for measurement of plasma concentrations of pegvaliase as specified in the SoA.

For participants randomized to active (pegvaliase) treatment, Part 1 blood samples for trough PK will be collected pre-dose (within 2 hours of dosing) at Day 1 and every 4 weeks for the first 24 weeks, followed by sampling every 8 weeks thereafter during Part 1. Intensive PK sampling will be performed at Week 73 and at dose escalations to 20, 40, and 60 mg/day. For intensive PK, samples will be taken at pre-dose (within 2 hours of dosing); at 2, 4, 8, and 12 hours (± 30 minutes) post-dose; and at 24 hours (± 2 hours) post dose. The 24-hour sample will be taken prior to the next daily dose. Extension Phase (Part 2) trough PK samples will be collected every 8 weeks.

For participants randomized to control (diet-only) treatment, there are no PK assessments in Part 1. In Part 2, the PK assessment schedule will mirror the Part 1 schedule performed for active participants, with trough PK samples collected pre-dose (within 2 hours of dosing) at Week 73 and every 4 weeks for the first 24 weeks and then every 8 weeks though the end of the study. Intensive PK sampling will be performed at Week 145 and for dose escalations to 20, 40, and 60 mg/day. For intensive PK, samples will be taken at pre-dose (within 2 hours of dosing); at 2, 4, 8, and 12 hours (± 30 minutes) post-dose; and at 24 hours (± 2 hours) post dose.

The PK parameters shown in Table 8.4.1 will be determined if feasible from the plasma concentration-time data for participants who have sufficient data for PK analysis (eg, at least 3 samples with measurable concentrations during the terminal log-linear phase for estimating

the terminal half-life and related parameters; at least 3 samples with measurable concentrations for other derived parameters).

Table 8.4.1: Derived Parameters for Pharmacokinetics Analysis

Parameter	Parameter Description
$AUC_{0-24hr,ss}$	Area under the plasma concentration-time curve from 0 to 24 hours at steady state, calculated using the linear trapezoidal rule
$C_{max,ss}$	Maximum observed plasma concentration within 24 hours post-dose at steady state
$T_{max,ss}$	Time to reach Cmax,ss
$C_{trough,ss}$	Trough observed plasma concentration prior to dosing at steady state
$t_{1/2}$	Terminal half-life, t1/2=ln2/λz where λz is the elimination rate constant
$CL_{ss/F}$	Apparent clearance of drug following subcutaneous injection at steady state
$V_{dz/F}$	Apparent volume of distribution based upon the terminal phase

The timing of sampling may be altered during the course of the study based on newly available data (eg, to obtain data closer to the time of peak plasma concentrations) to ensure appropriate monitoring. Instructions for the collection and handling of biological samples will be provided by the sponsor. The actual date and time (24-hour clock time) of each sample will be recorded.

Plasma samples collected for analyses of pegvaliase concentration may also be used for exploratory safety or efficacy analyses during or after the study.

Genetic analyses will not be performed on these plasma samples.

8.5 Pharmacodynamics

Blood Phe and Tyrosine (Tyr) will be measured by validated assays (see Section 8.1.1 and Section 1.3).

8.6 Biomarkers and Genetic Assessment

8.6.1 PAH Genotyping

PAH genotype testing should be performed where allowed by local health authorities and the EC/IRB.

8.6.2 Blood for Exploratory Analyses

As an exploratory analysis, blood will be collected to evaluate biochemical, molecular, and cellular aspects of PKU and to develop the assays used for these evaluations. The blood samples reserved for exploratory analyses are collected at the time points indicated in the SoA (see Section 1.3).

For each portion of blood samples reserved for protocol-specified analyses, there may be multiple sample aliquots. Once samples have been successfully analyzed during the study per protocol, unused sample portions may be used during the study and afterwards for assay development and other purposes stated in this section.

8.7 Immunogenicity Assessments

Sampling for immunogenicity testing will be performed routinely throughout the study to provide a comprehensive assessment of the immune response against pegvaliase. Antibodies against pegvaliase will be evaluated in serum samples collected from all participants according to the SoA (Section 1.3). Additionally, serum samples should be collected at the final visit from participants who discontinued study treatment or were withdrawn from the study.

Routine immunogenicity testing for anti-drug antibodies will be performed using validated semi-quantitative assays used in previous Phase 3 studies. Titer and incidence of positivity for each of the following analytes will be reported: total anti-pegvaliase antibodies (TAb), anti-PAL IgG, anti-PAL IgM, anti-PEG IgM, anti-PEG IgG, and pegvaliase-neutralizing antibodies (NAb). In the event of a hypersensitivity reaction visit (HRV) only, assessment of anti-pegvaliase IgE antibodies will be performed using an ImmunoCAP assay after depletion of IgG and IgM.

8.8 Health Economics/Medical Resource Utilization and Health Economics

Health Economics/Medical Resource Utilization and Health Economics parameters are not evaluated in this study.

9 STATISTICAL CONSIDERATIONS

9.1 Statistical Hypotheses

The study hypothesis is that participants receiving pegvaliase will show a greater reduction in blood Phe following 72 weeks on study compared to the diet-only control group.

Let μ_a and μ_b denote the mean change from baseline in blood Phe following 72 weeks on study in the pegvaliase and diet-only group, respectively. The null and alternative hypotheses are:

H_0: $\mu_a = \mu_b$

H_1: $\mu_a \neq \mu_b$

The hypotheses will be tested at significance level of 0.05 (two-sided).

9.2 Sample Size Determination

Approximately 54 participants will be enrolled into the study and randomized to the active (pegvaliase) or control (diet-only) group in 2:1 ratio. Assuming a mean (SD) reduction in Phe following 72 weeks on study of 640 (570) µmol/L in the pegvaliase group and 100 (250) µmol/L in the diet-only group, a total sample size of 54 participants will provide more than 90% power to detect a treatment difference, based on the two-sample T-test with unequal variance and a significance level of 0.05 (two sided). The assumption of treatment effect is based on clinical data from previous pegvaliase studies and a PKU registry study. See the Statistical Analysis Plan (SAP) for additional details on the sample size calculation.

Approximately 72 participants will be screened for study participation such that approximately 54 participants will be enrolled in the study.

Note: Potential participants who are screened for the purpose of determining eligibility for the study, but do not participate in the study, are not considered enrolled.

9.3 Populations for Analysis

The analysis populations are defined in Table 9.3.1.

Table 9.3.1: Analysis Populations

Population	Description
All Enrolled	All participants (or their parent/guardians, if applicable) who agree to participate in the clinical study following completion of the informed consent process and determination of study eligibility.
ITT Population	All participants who are randomized.
Safety Population	All participants who received study treatment (pegvaliase or diet-only management of PKU).
Per Protocol Population	All participants in the ITT population who are compliant with the protocol with no major protocol deviations impacting the efficacy analysis. Major protocol deviations impacting efficacy analysis will be defined and documented in the SAP.
PK Population	All participants with at least one PK measurement

9.4 Statistical Analysis

The SAP will be finalized prior to database lock (DBL) and will include a more technical and detailed description of the statistical analyses described in this section. This section is a summary of the planned statistical analyses of the most important endpoints, including primary and key secondary endpoints.

A clinical pharmacology analysis plan will also be finalized prior to DBL.

Note: If discrepancies exist between the text of the statistical analysis as planned in the protocol, and the SAP, the SAP will prevail.

9.4.1 General Considerations

Descriptive analyses will be performed to gain an understanding of the qualitative and quantitative nature of the data collected and the characteristics of the sample collected. Continuous variables will be reported as mean, standard deviation (SD), median, minimum (min) and maximum (max). Categorical variables will be presented using absolute and relative frequency counts and percentage.

All generation of tables, listings and figures described in the SAP will be performed using SAS® version 9.4 or higher.

Full details on handling missing data and the corresponding analysis methods will be described in detail in the SAP.

9.4.2 Primary Endpoints

The primary efficacy endpoint is the change from baseline in blood Phe following 72 weeks on study. Baseline blood Phe is defined as the mean of 2 pre-treatment (Screening/Run-in)

measurements to be performed 7 to 10 days apart during the Screening/Run-in period. The blood Phe measurement following 72 weeks on study is defined as the average of the Week 69 and Week 73 blood Phe measurements.

Change from baseline will be compared between participants in the active (pegvaliase) arm and the control (diet-only) arm. The primary efficacy endpoint will be evaluated using an Analysis of Covariance (ANCOVA) model, which will include baseline blood Phe as covariate and treatment group as factor, with missing data handled by multiple imputation method. The least square (LS) mean and 95% confidence interval will be calculated for the treatment difference between pegvaliase and diet-only arms following 72 weeks on study. Additional imputation method may be conducted as sensitivity analysis, further details will be provided in the SAP.

9.4.3 Secondary Endpoints

The secondary endpoints are as follows:

- Change from baseline in total dietary protein intake (ie, medical food and/or intact food). Baseline total protein intake is defined as the mean total dietary protein intake calculated from the 3 separate 3-day diet diaries collected during Screening/Run-in. The total dietary protein intake following 72 weeks on study is defined as the mean total dietary protein intake calculated from the diet diaries collected at the Week 69 and Week 73 visits. Total dietary protein is measured in grams/kilogram (g/kg).
- Plasma sample for trough PK

Descriptive summary will be provided. Full details will be provided in the SAP.

9.4.4 Exploratory Endpoints

Refer to the SAP for a discussion of analysis for exploratory endpoints.

9.4.5 Other Safety Analyses

Safety analyses will be performed on the Safety Population. AEs will be coded using the most recent version of the Medical Dictionary for Regulatory Activities (MedDRA). The incidence of treatment-emergent AEs, SAEs, and AESIs will be summarized by MedDRA system organ class, preferred term, relationship to study drug, and severity. Participants with AEs that result in study drug discontinuation, dosing interruption, or dose level reduction will be tabulated. A by-participant listing of all AEs will be provided.

Clinical laboratory data will be summarized descriptively at baseline and post-baseline visits. Shift tables will be created to summarize the change in CTCAE grade from baseline to worst post-baseline value. Clinically significant laboratory abnormalities reported as AEs will be

summarized. Descriptive statistics for vital signs, physical examination results, ECG test results, and immunogenicity test results will also be provided. Details of statistical methods will be provided in the SAP.

9.5 Interim Analyses

No interim analyses are planned for this study.

9.6 Data Monitoring Committee (DMC)

The independent Data Monitoring Committee (DMC) will act in an advisory capacity to BioMarin to monitor participant safety and the efficacy of pegvaliase in participants who participate in Study 165-306. The DMC responsibilities may include:

- Review the study protocol, informed consent and assent documents, and plans for data monitoring
- Evaluate the progress of the trial, study data quality, timeliness, participant recruitment, accrual and retention, participants' risk versus benefit, and other factors that could affect the study outcome
- Consider relevant information that may have an impact on the safety of the participants or the ethics of the study
- Protect the safety of the study participants in accordance with the stopping rules as defined in study protocol
- Make recommendations to BioMarin concerning continuation, termination or other modifications of the study based on their observations of the study

Further details are provided in the DMC Charter.

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 Appendix 1: Regulatory, Ethical, and Study Oversight Considerations

10.1.1 Regulatory and Ethical Considerations

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

- Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines
- Principles of Good Clinical Practice as described in the US Code of Federal Regulations and the International Conference on Harmonisation Guidelines
- Applicable laws and regulations

The protocol, protocol amendments, ICF, Investigator Brochure, and other relevant documents (eg, advertisements) as applicable must be submitted, reviewed, and approved by the IRB/IEC before the study is initiated.

Any amendments to the protocol will require IRB/IEC approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study participants.

The investigator will be responsible for the following:

- Providing written summaries of the status of the study to the IRB/IEC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/IEC
- Notifying the IRB/IEC of SAEs or other significant safety findings as required by IRB/IEC procedures
- Providing oversight of the conduct of the study at the site and adherence to requirements of 21 CFR, ICH guidelines, the IRB/IEC, European regulation 536/2014 for clinical studies (if applicable), and all other applicable local regulations

BioMarin will pay the full costs of the study-related tests, procedures, and treatments set forth in this protocol. In addition, after IRB/IEC approval, BioMarin may reimburse the reasonable cost of travel for study-related visits in accordance with BioMarin's travel and reimbursement policy.

The investigator should contact BioMarin immediately upon notification that a study participant has been injured by the study drug or by procedures performed as part of the study. Any participant who experiences a study-related injury should be instructed by the

investigator to seek immediate medical treatment at a pre-specified medical institution if possible, or at the closest medical treatment facility if necessary. The participant should be given the name of a person to contact to seek further information about, and assistance with, treatment for study-related injuries. BioMarin or the institution may pay for reasonable and necessary medical services to treat the injuries caused by the study drug or study procedures. In some jurisdictions, BioMarin is obligated by law to pay for study-related injuries. If this is the case, BioMarin will comply with the law. BioMarin will not pay for any hospitalizations, tests, or treatments for medical problems of any sort related solely to the study participant's primary disease or any concurrent disease and that are unrelated to the study.

Significant Noncompliance Reporting: Any deviation or series of deviations from the Protocol, ICH GCP, regulatory, and/or legal requirements that is likely to affect to a significant degree the safety or rights of a participant; the stability, quality, and/or control of study drug; or the quality or integrity of the study data shall be reported in writing to BioMarin within one (1) business day upon becoming aware of any suspicion or discovery of Significant Noncompliance.

10.1.2 Statement of Investigator and Financial Disclosure

Prior to beginning the study, the investigator at each site must provide to sponsor or designee a fully executed and signed Statement of Investigator (SOI) form. A US Food and Drug Administration (FDA) Form FDA 1572 serves as an acceptable SOI form. If Form FDA 1572 may not be used in a particular region, the investigator must provide a fully executed SOI on the form provided by the sponsor. All investigators and sub-investigators must be listed on Form FDA 1572 or its equivalent SOI. Financial Disclosure Forms must be completed with sufficient, accurate financial information as requested for all investigators and sub-investigators listed on the Form FDA 1572 or SOI who will be directly involved in the treatment or evaluation of participants in this study. The Financial Disclosure Forms allow the sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities. Investigators must submit an updated Financial Disclosure Form to BioMarin should their response to any questions within the form or disclosure status change during the period of the study and for one year after study completion.

In accordance with FDA Form 1572 and/or principles of ICH GCP, the investigator will ensure that:

- He or 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 participants.

- He or she will personally conduct or supervise the study.
- He or she will inform any potential participants, 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 CFR Part 50 and/or ICH Sections 2.9 and 4.8 are met. As well, he or she will ensure that IRB/IEC review and approval in 21 CFR Part 56 and/or ICH Section 2.6 are met.
- He or she will report to the sponsor adverse experiences that occur in the course of the investigation in accordance with 21 CFR 312.64 and/or ICH Section 4.11.
- He or she has read and understands the information in the IB, including potential risks and side effects of the drug.
- His or her staff and all persons who assist in the conduct of the study are informed about their obligations in meeting the above commitments.
- Adequate and accurate records in accordance with 21 CFR 312.62 and/or ICH Section 4.9 are kept, and those records are available for inspection in accordance with 21 CFR 312.68 and/or ICH Section 4.9.7.
- The IRB/EC/REB complies with the requirements of 21 CFR Part 56, ICH Section 3.0, and other applicable regulations, and conducts initial and ongoing reviews and approvals of the study. He or she will also ensure that any change in research activity and all problems involving risks to human participants or others are reported to the IRB/EC/REB. Additionally, he or she will not make any changes in the research without IRB/EC/REB approval, except where necessary to eliminate apparent immediate hazards to human participants.
- He or she agrees to comply with all other requirements regarding the obligations of clinical Investigators and all other pertinent requirements in 21 CFR Part 312 and/or ICH E6 R2.

10.1.3 Informed Consent Process

The investigator or his/her representative will explain the nature of the study to the participant or his/her legally authorized representative and answer all questions regarding the study.

Participants must be informed that their participation is voluntary. Participants or their legally authorized representative (as determined by the laws of the jurisdiction in which the study is being conducted) will be required to sign a statement of informed consent that meets the regulatory requirements of the country or region where the participant consents, such as 21 CFR 50 (US FDA), ICH guidelines, privacy requirements including but not limited to the Health Insurance Portability and Accountability Act (HIPAA) requirements and the General Data Protection Regulation (where applicable), and the IRB/IEC or study center. If there is no applicable law addressing the issue of who may be a legally authorized representative, a

legally authorized representative will be an individual recognized by institutional policy as acceptable for providing consent on behalf of the prospective participant to the participant's participation in the procedure(s) involved in the research.

The medical record must include a statement that written informed consent was obtained before any study specific procedures were initiated and the date the written consent was obtained. The authorized person obtaining the informed consent must also sign the ICF.

Participants must be re-consented to the most current version of the ICF(s) during their participation in the study. Participants who reach the age of majority in their country while the study is ongoing will be asked to provide their own written consent again upon reaching the legal age of majority.

The ICF will contain a separate section that addresses the use of remaining samples for optional additional research. The investigator or authorized designee will explain to each participant the objectives of the additional research. Participants will be told that they are free to refuse to participate and may withdraw their consent at any time and for any reason during the storage period. A separate signature will be required to document a participant's agreement to allow any remaining samples to be used for additional research.

A copy of the ICF(s) must be provided to the participant or the participant's legally authorized representative.

A participant who is rescreened is not required to sign another ICF if the rescreening occurs during the Screening/Run-in period.

10.1.4 Data Protection

Measures will be taken to ensure the privacy of participant data, including the use of participant numbers on eCRFs. A list linking participant identification numbers with participant names and other personal information will be kept in a secure place, separate from the participants' medical records. BioMarin will follow all applicable regulations and guidelines in the relevant locality, country, and/or region to protect the privacy of participant data.

Participants will be assigned a unique identifier by the sponsor. Any participant records or datasets that are transferred to the sponsor will contain the identifier only; participant names or any information which would make the participant identifiable will not be transferred.

The participant must be informed that his/her personal study-related data will be used by the sponsor in accordance with local data protection law. The level of disclosure must also be

explained to the participant, who will be required to give consent or be given notice of the legal bases for their data to be used as described in the informed consent.

The participant must be informed that his/her medical records may be examined by authorized personnel appointed by the sponsor, by appropriate IRB/IEC members, and by inspectors from regulatory authorities.

10.1.5 Committee Structures

The following advisory and assurance activities will be implemented to support the study: monitoring committees, clinical trial disclosure, and study monitoring.

10.1.5.1 Data Monitoring Committee (DMC)

An external independent Data Monitoring Committee (DMC) will be responsible for monitoring participant safety and efficacy of pegvaliase. The DMC consisting of five members, who are each independent of the conduct of studies in the BioMarin BMN 165 (pegvaliase) development program, and are experts in the fields of PKU, clinical immunology, neuropsychology, pharmacovigilance, and biostatistics. DMC responsibilities are described in Section 9.6.

10.1.5.2 Internal Safety Data Review Board

The Internal Safety Data Review Board, consisting of a representative from BioMarin clinical sciences, safety, and biostatistics, will review all cases of potential anaphylaxis to determine which of these events meet the criteria for an acute systemic hypersensitivity reaction as summarized in Appendix 5, Section 10.5.2.

10.1.6 Dissemination of Clinical Study Data

The posting of study information and study results will comply with applicable national regulatory requirements and BioMarin's data sharing policy available at https://www.biomarin.com/data-request-form/.

10.1.7 Data Quality Assurance

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

Sites will enter study data into eCRFs in the study EDC system. Data Quality Control will be performed by BioMarin Clinical Data Management or a designee through implementation of

quality control checks specified in the study data management plan and edit check specifications.

Study monitors will perform ongoing source data verification to confirm that data entered into the eCRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of participants are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements. Qualified individuals designated by BioMarin will monitor all aspects of the study according to 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 participants, 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 BioMarin or its designees.

The investigator must retain all study records required by BioMarin and by the applicable regulations in a secure and safe facility. The investigator must notify BioMarin 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, that is, 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, participant 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) at least 2 years have elapsed since the formal discontinuation of clinical development of the IP. The investigator/institution should retain participant identifiers for at least 15 years after the completion or discontinuation of the study. Participant 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 a BioMarin agreement. BioMarin must be notified and will assist with retention should the investigator/institution be unable to continue maintenance of participant files for the full 15 years. It is the responsibility of BioMarin to inform the investigator/institution as to when these documents no longer need to be retained.

Representatives of BioMarin or designees may conduct an audit of a site at any time before, 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 inspection of the study. If informed of such an inspection, the investigator should notify BioMarin immediately. The investigator will ensure that the auditors/inspectors have access to the clinical supplies, site facilities, original source documentation, and all study files.

10.1.8 Source Documents

Source documents provide evidence for the existence of the participant and substantiate the integrity of the data collected. Source documents are filed at the investigator's site.

Data entered in the eCRF that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The investigator may need to request previous medical records or transfer records, depending on the study. Also, current medical records must be available.

The definition of what constitutes source data can be found in the Monitoring Plan.

10.1.9 Study and Site Start and Closure

First Act of Recruitment

The study start date is the date on which the clinical study will be open for recruitment of participants.

The first act of recruitment is the first site open and will be the study start date.

Study/Site Termination

The sponsor reserves the right to close the site or terminate the study at any time for any reason at the sole discretion of the sponsor. Sites will be closed upon study completion. A site is considered closed when all required documents and unused study supplies have been collected and a site closure visit has been performed.

Reasons for the early closure of a site by the sponsor may include but are not limited to:

- Failure of the investigator to comply with the protocol, the requirements of the IRB/IEC or local health authorities, the sponsor's procedures, or GCP guidelines
- Inadequate recruitment of participants by the investigator
- Discontinuation of further study treatment development

If the study is prematurely terminated or suspended, the sponsor shall promptly inform the investigators, the IECs/IRBs, the regulatory authorities, and any contract research

organization(s) used in the study of the reason for termination or suspension, as specified by the applicable regulatory requirements. The investigator shall promptly inform the participant(s) and should assure appropriate participant therapy and/or follow-up.

10.1.10 Publication Policy

The results of this study may be published or presented at scientific meetings. If this is foreseen, the investigators agree to submit all manuscripts or abstracts to the sponsor at least forty-five (45) days before submission or as agreed in the Clinical Trial Agreement between BioMarin and the investigator/institution. This allows the sponsor to protect proprietary information and to provide comments.

The sponsor will comply with the requirements for publication of study results. In accordance with standard editorial and ethical practice, the sponsor will generally support publication of multicenter studies only in their entirety and not as individual site data. In this case, a coordinating investigator will be designated by mutual agreement.

BioMarin recognizes the importance of communicating medical study data and therefore encourages the publication of these data 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 may be described in the Clinical Trial Agreement between BioMarin and the investigator/institution. Consideration for authorship of all publications will be based on mutual agreement and compliance with the Uniform Requirements for Manuscripts Submitted to Biomedical Journals (“Uniform Requirements”) of the International Committee of Medical Journal Editors (ICMJE) (http://www.icmje.org/about-icmje/faqs/icmje-recommendations/) and good publication practices (GPP).

10.2 Appendix 2: Clinical Laboratory Tests

The tests detailed in Table 10.2.1 will be performed by the central laboratory.

Protocol-specific requirements for inclusion or exclusion of participants are detailed in Section 5 of the protocol.

Additional tests may be performed at any time during the study as determined necessary by the investigator or required by local regulations.

Investigators must document their review of each laboratory safety report.

Table 10.2.1: Clinical Laboratory Tests

Laboratory Test	Parameters		
Chemistry	Albumin Alkaline Phosphatase ALT (SGPT) AST (SGOT) Bicarbonate Direct bilirubin Total bilirubin	BUN Calcium Total cholesterol CO_2 Creatinine Creatine phosphokinase Glucose (fasting)	GGT LDH Phosphorus Potassium Total protein Sodium Uric acid
Hematology	Hemoglobin Hematocrit	WBC count RBC count	Platelet count Differential cell count
Urinalysis	Appearance Color pH Specific gravity Ketone Protein	Glucose Bilirubin Nitrite Urobilinogen Hemoglobin	Urinary albumin/creatinine ratio [a] Urinary microscopy
Pregnancy testing	Refer to Section 8.2.5		
Complement panel	C3 C4		
Other tests conducted during the study (refer to the SoA for timing)	Phenylalanine Tyrosine hs-CRP Serum tryptase level Serum cortisol [b] Erythrocyte sedimentation rate [c] Urinary N-methylhistamine		

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; C3, C4, complement components 3, 4; CO2, carbon dioxide; ESR, Erythrocyte sedimentation rate; GGT, gamma-glutamyltransferase; hs-CRP, high sensitivity C-reactive protein; LDH, lactate dehydrogenase; RBC, red blood cell; SGOT, serum glutamic-oxaloacetic transaminase; SGPT, serum glutamic-pyruvic transaminase; WBC, white blood cell.

[a] It is recommended that urine samples be obtained as a first or second morning void. Participants with a confirmed urine/albumin creatinine ratio of ≥ 100 mg/g should be referred to a nephrologist for consultation if results were within normal range at Baseline. Participants who had elevated results at Baseline followed by a confirmed subsequent increase of 100-200 mg/g from Baseline should also be referred to a nephrologist for consultation.

[b] Serum cortisol samples should be taken before study drug administration in the morning before 10:00 am. A home healthcare nurse may collect samples for participant convenience. If 2 results are low and abnormal, the participant will be asked to perform additional sampling for serum cortisol and a low-dose conventional ACTH stimulation test or the ACTH test method based on the clinical practice at the site.

[c] ESR will be performed at a local laboratory.

10.3 Appendix 3: Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting

10.3.1 Definition of an Adverse Event

AE Definition
• An AE is any untoward medical occurrence in a patient or clinical study participant, temporally associated with the use of study treatment, whether or not considered related to the study treatment. • NOTE: An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of study treatment.

Events Meeting the AE Definition
• Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (eg, ECG, radiological scans, vital signs measurements), including those that worsen from baseline, considered clinically significant in the medical and scientific judgment of the investigator (ie, not related to progression of underlying disease). • Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition. • New conditions detected or diagnosed after study treatment administration. • Signs, symptoms, or the clinical sequelae of a suspected drug-drug interaction. • Signs, symptoms, or the clinical sequelae of a suspected overdose of either study treatment or a concomitant medication. Overdose per se will not be reported as an AE/SAE unless it is an intentional overdose taken with possible suicidal/self-harming intent. Such overdoses should be reported regardless of sequelae. • "Lack of efficacy" or "failure of expected pharmacological action" per se will not be reported as an AE or SAE. Such instances will be captured in the efficacy assessments. However, the signs, symptoms, and/or clinical sequelae resulting from lack of efficacy will be reported as AE or SAE if they fulfill the definition of an AE or SAE.

Events Not Meeting the AE Definition
• Any clinically significant abnormal laboratory findings or other abnormal safety assessments which are associated with the underlying disease, unless judged by the investigator to be more severe than expected for the participant's condition. • The disease/disorder being studied or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the participant's condition. • Medical or surgical procedure (eg, endoscopy, appendectomy): the condition that leads to the procedure is the AE. • Situations in which an untoward medical occurrence did not occur (social and/or convenience admission to a hospital).

Events Not Meeting the AE Definition
• Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen. • Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline. • Planned hospitalization for childbirth (eg, scheduled Caesarean section).

10.3.2 Definition of an SAE

If an event is not an AE per the definition above, then it cannot be an SAE even if serious conditions are met (eg, hospitalization for signs/symptoms of the disease under study, death due to progression of disease).

A SAE is defined as any untoward medical occurrence that, at any dose:
a. Results in death
b. Is life-threatening The term "life-threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event. It does not refer to an event that hypothetically might have caused death if it were more severe.
c. Requires inpatient hospitalization or prolongation of existing hospitalization In general, hospitalization signifies that the participant has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious.
d. Results in persistent disability/incapacity The term disability means a substantial disruption of a person's ability to conduct normal life functions. This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (eg, sprained ankle) which may interfere with or prevent everyday life functions but do not constitute a substantial disruption.
e. Is a congenital anomaly/birth defect in the offspring of a study participant
f. Other situations/Important Medical Events: Medical or scientific judgment should be exercised in deciding whether SAE reporting is appropriate in other situations such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the participant or may require medical or surgical treatment to prevent one of the other outcomes listed in the above definition. These events should usually be considered serious. Examples of such events include invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

10.3.3 Recording and Follow-Up of AEs and/or SAEs

AE and SAE Recording

- When an AE/SAE occurs, it is the responsibility of the investigator to review all documentation (eg, hospital progress notes, laboratory reports, and diagnostics reports) related to the event.
- The investigator or qualified designee will then record all relevant AE/SAE information in the eCRF.
- It is **not** acceptable for the investigator to send photocopies of the participant's medical records to BioMarin in lieu of completion of the AE/SAE eCRF page.
- There may be instances when copies of medical records for certain cases are requested by BioMarin. In these cases, all participant identifiers, with the exception of the participant number, will be redacted on the copies of the medical records before submission to BioMarin.
- The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. Whenever possible, the diagnosis (not the individual signs/symptoms) will be documented as the AE/SAE.

Assessment of Intensity

The investigator will make an assessment of intensity for each AE and SAE reported during the study and assign it to 1 of the following categories (as defined in Common Terminology Criteria for Adverse Events [CTCAE] version 5.0). The investigator should only record the single highest grade of intensity for each AE and SAE in the eCRF. Adverse events that do not have a corresponding CTCAE term will be assessed according to the general guidelines for grading:

- Grade 1 (Mild): asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated
- Grade 2: (Moderate): minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL) (eg, preparing meals, shopping for groceries or clothes, using the telephone, managing money)
- Grade 3 (Severe): medically significant but not immediately life-threatening: hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL (eg, bathing, dressing/undressing, feeding oneself, using the toilet, taking medications, not bedridden)
- Grade 4: Life threatening consequences; urgent intervention indicated
- Grade 5: Death related to AE

An event is defined as "serious" when it meets at least 1 of the predefined outcomes as described in the definition of an SAE, NOT when it is rated as severe.

Assessment of Causality

- The investigator is obligated to assess the relationship between study treatment and each occurrence of each AE/SAE.
- An assessment of "related" means that there are facts, evidence, and/or arguments to suggest a causal relationship such that a relationship cannot be ruled out.
- The investigator will use clinical judgment to determine the relationship.

Assessment of Causality
• Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to study treatment administration will be considered and investigated. • For each AE/SAE, the investigator must document in the medical notes that he/she has reviewed the AE/SAE and has provided an assessment of causality. • There may be situations in which an SAE has occurred, and the investigator has minimal information to include in the initial report to BioMarin. However, it is very important that the investigator always make an assessment of causality for every event before the initial transmission of the SAE data to BioMarin. • The investigator may change his/her opinion of causality in light of follow-up information and send a SAE follow-up report with the updated causality assessment.

Follow-up of AEs and SAEs
• The investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by BioMarin to elucidate the nature and/or causality of the AE or SAE as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals. • If a participant dies during participation in the study or during a recognized follow-up period, the investigator will provide a copy of any post-mortem findings including histopathology. • New or updated information will be recorded in the originally completed eCRF. • The investigator will submit any updated SAE data to the sponsor within 24 hours of receipt of the information.

10.3.4 Reporting of SAEs and Events of Special Interest

SAE/AESI Reporting to BioMarin Pharmacovigilance (BPV) via an Electronic Data Collection Tool
• The primary mechanism for reporting an SAE/AESI to BPV will be the electronic data collection tool. AESI reporting will follow the SAE reporting requirements. • If the electronic system is unavailable, then the site will use the paper SAE data collection tool (see next section) in order to report the event within 24 hours. • The site will enter the SAE/AESI data into the electronic system as soon as it becomes available. • After the study is completed at a given site, the electronic data collection tool will be taken off-line to prevent the entry of new data or changes to existing data. • If a site receives a report of a new SAE/AESI from a study participant or receives updated data on a previously reported SAE/AESI after the electronic data collection tool has been taken off-line, then the site can report this information on a paper SAE form (see next section) or to BPV by telephone.

SAE/AESI Reporting to BPV via Paper CRF
• Facsimile transmission or email of the paper CRF (pregnancy form, pregnancy outcome, or SAE form is EDC unavailable) is the preferred method to transmit this information to BPV. • In rare circumstances and in the absence of facsimile equipment, notification by telephone is acceptable with a copy of the paper CRF sent by overnight mail or courier service. • Initial notification via telephone does not replace the need for the investigator to complete and sign the CRF pages within the designated reporting time frames.

Contact information for BioMarin Pharmacovigilance is as follows:

BioMarin Pharmaceutical Inc.
Address 105 Digital Drive
Novato, CA 94949
Phone: PI
Fax:
E-mail: drugsafety@bmrn.com

The Investigator is encouraged to discuss with the medical monitor any AEs for which the issue of seriousness is unclear or questioned. Contact information for the medical monitor is provided on the signature page at the front of this protocol.

Name: PI RN, MS
Address: 105 Digital Drive
Novato, CA 94949 USA
Phone: PI
Fax:
E-mail:

10.4 Appendix 4: Contraceptive and Barrier Guidance

Definitions:

Woman of Childbearing Potential (WOCBP)

A woman is considered fertile following menarche and until becoming post-menopausal unless permanently sterile (see below).

If fertility is unclear (eg, amenorrhea in adolescents or athletes) and a menstrual cycle cannot be confirmed before first dose of study treatment, additional evaluation should be considered.

Women in the following categories are not considered WOCBP:

- Premenarchal
- Premenopausal female with 1 of the following:
    - Documented hysterectomy
    - Documented bilateral salpingectomy
    - Documented bilateral oophorectomy

  For individuals with permanent infertility due to a medical cause other than the above (eg, mullerian agenesis, androgen insensitivity), investigator discretion should be applied in determining study eligibility.

  Note: Documentation can come from the site personnel's review of the participant's medical records, medical examination, or medical history interview.
- Postmenopausal female
    - A postmenopausal state is defined as no menses for 12 months without an alternative medical cause.
        - A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a postmenopausal state in women not using hormonal contraception or hormonal replacement therapy (HRT). However, in the absence of 12 months of amenorrhea, confirmation with more than one FSH measurement is required.
    - Females on HRT whose menopausal status is in doubt will be required to use one of the non-estrogen hormonal highly effective contraception methods if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of postmenopausal status before study enrollment.

Contraception Guidance:

If sexually active, must be willing to use two acceptable methods of contraception while participating in the study and 4 weeks after the last dose of pegvaliase.

10.5 Appendix 5: Anaphylaxis Criteria

For the purposes of this study, the term anaphylaxis refers to the important identified risk of acute systemic hypersensitivity reaction. An acute systemic hypersensitivity reaction is one that is consistent with the NIAID/FAAN definition as assessed by BioMarin using the criteria listed below in Appendix 5, Section 10.5.1 and Appendix 5, Section 10.5.2.

10.5.1 Sampson's Anaphylaxis Criteria

According to the National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network (NIAID/FAAN) Second Symposium on the definition and management of anaphylaxis, anaphylaxis is highly likely when any one of the following 3 criteria are fulfilled:

1. Acute onset of an illness (minutes to several hours) with involvement of the skin, mucosal tissue, or both (eg, generalized hives, pruritus or flushing, swollen lips-tongue-uvula)

   AND AT LEAST ONE OF THE FOLLOWING:

   a. Respiratory compromise (eg, dyspnea, wheeze-bronchospasm, stridor, reduced peak expiratory flow [PEF], hypoxemia)

   b. Reduced blood pressure (BP) or associated symptoms of end-organ dysfunction (eg, hypotonia [collapse], syncope, incontinence)

2. Two or more of the following that occur rapidly after exposure *to a likely allergen for that patient* (minutes to several hours):

   a. Involvement of the skin-mucosal tissue (eg, generalized hives, itch-flush, swollen lips-tongue-uvula)

   b. Respiratory compromise (eg, dyspnea, wheeze-bronchospasm, stridor, reduced PEF, hypoxemia)

   c. Reduced BP or associated symptoms (eg, hypotonia [collapse], syncope, incontinence)

   d. Persistent gastrointestinal symptoms (eg, crampy abdominal pain, vomiting)

3. Reduced BP after exposure to *known* *allergen for that patient* (minutes to several hours):
    a. Infants and children: low systolic blood pressure (age specific) or greater than 30% decrease is systolic BP
    b. Adults: systolic BP of less than 90 mmHg or greater than 30% decrease from that person's baseline.

Source: Sampson 2006.

10.5.2 Acute Systemic Hypersensitivity Reaction

An acute systemic hypersensitivity reaction is a Type III acute systemic allergic reaction. An acute systemic hypersensitivity reaction has a similar clinical presentation to an anaphylactic reaction, but only meets the definition if the italicized criteria below are met in addition to the criteria outlined in Sampson's criteria (see Appendix 5, Section 10.5.1, Sampson's Criteria):

Sampson Criteria Number	Sampson Criteria Wording With Additional Criteria Necessary to Meet Acute Systemic Hypersensitivity Reaction Criteria Shown in Italics
1	Acute onset of an illness (minutes to several hours) with involvement of the skin, mucosal tissue, or both (eg, generalized hives, pruritus or flushing, swollen lips-tongue-uvula). *Must be generalized skin reaction to meet criteria*.
1a	Respiratory compromise (eg, dyspnea, wheeze-bronchospasm, stridor, reduced PEF, hypoxemia). *Respiratory compromise is demonstrated by oxygen desaturation below 88% on room air.*
1b	Reduced BP or associated symptoms of end-organ dysfunction (eg, hypotonia [collapse], syncope, incontinence). *Systolic BP less than 90 mmHg (adults) or 70 mmHg (children) or greater than 30% decrease from baseline*.
2d	Persistent gastrointestinal symptoms (eg, crampy abdominal pain, vomiting). *Gastrointestinal symptoms are persistent with multiple episodes that continue for several hours or more*.

10.5.3 Anaphylaxis Analysis

During the initial BLA for Palynziq in the US, the FDA proposed that anaphylaxis should be defined for labelling purposes using the methodology developed by FDA's Division of Pulmonary, Allergy, and Rheumatology Products (DPARP). As a result, in the study analyses for 165-306 anaphylaxis, including derivation of the incidence and event rates, data will be presented using both BioMarin's definition of an acute systemic hypersensitivity reaction described above and the FDA's assessment methodology, ie, a case will be counted as an anaphylaxis event if it meets any of the following criteria:

- Clinical manifestations consistent with NIAID/FAAN criterion #1 (skin involvement + respiratory or cardiovascular compromise); or
- Verbatim reports of anaphylaxis/anaphylactic reaction (or synonyms), even if NIAID/FAAN criteria were not met; or
- Cases involving participant injection of epinephrine alone, even if the participant did not experience signs/symptoms necessary to meet NIAID/FAAN criteria.

Further details are provided in the SAP.

10.6 Appendix 6: Medical Device Adverse Events, Adverse Device Effects (ADEs), Serious Adverse Events (SAEs), and Device Deficiencies: Definition and Procedures for Recording, Evaluating, Follow-Up, and Reporting

Medical Device Adverse Event reporting applies only to US sites. The definitions and procedures detailed in this appendix are in accordance with ISO 14155.

Both the investigator and the sponsor will comply with all local medical device reporting requirements.

The detection and documentation procedures described in this protocol apply to all sponsor medical devices provided for use in the study. For this study, prefilled syringes are provided (see Section 6.1.1).

10.6.1 Definition of AE and ADE

AE and ADE Definition
• An AE is defined as any untoward medical occurrence, unintended disease or injury, or untoward clinical signs (including abnormal laboratory finding) in study participants, users, or other persons, whether or not related to the investigational medical device. This definition includes events related to the investigational medical device or comparator and events related to the procedures involved except for events in users or other persons, which only include events related to investigational devices. • An adverse device effect (ADE) is defined as an adverse event related to the use of an investigational medical device. This definition includes any adverse events resulting from insufficient or inadequate instructions for use, deployment, implantation, installation, or operation, or any malfunction of the investigational medical device as well as any event resulting from use error or from intentional misuse of the investigational medical device.

10.6.2 Definition of SAE, SADE, and USADE

If an event is not an AE per definition above, then it cannot be an SAE even if serious conditions are met (eg, hospitalization for signs/symptoms of the disease under study, death due to disease progression).

An SAE is an AE that:

- Led to death
- Led to serious deterioration in the health of the participant resulting in either:
  1. A life-threatening illness or injury. The term 'life-threatening' in the definition of 'serious' refers to an event in which the participant was at risk of death at the time of the event. It does not refer to an event which hypothetically might have caused death if it were more severe.
  2. A permanent impairment of a body structure or a body function
  3. Inpatient or prolonged hospitalization, planned hospitalization for a pre-existing condition, or a procedure required by the protocol, without serious deterioration in health, is not considered an SAE.
  4. Medical or surgical intervention to prevent life-threatening illness or injury or permanent impairment to a body structure or a body function
- Led to fetal distress, fetal death, or a congenital abnormality or birth defect

SADE definition:

- A SADE is defined as an adverse device effect that has resulted in any of the consequences characteristic of a serious adverse event.

USADE definition

- A USADE is a serious adverse device effect which by its nature, incidence, severity, or outcome has not been identified in the current version of the risk analysis report.

10.6.3 Definition of Device Deficiency

Device deficiency definition

- A device deficiency is an inadequacy of a medical device with respect to its identity, quality, durability, reliability, safety, or performance. Device deficiencies include malfunctions, use errors, and inadequate labeling.

10.6.4 Recording and Follow-Up of AE and/or SAE and Device Deficiencies

AE, SAE, and Device Deficiency Recording

- When an AE/SAE/device deficiency occurs, it is the responsibility of the investigator to review all documentation (e.g., hospital progress notes, laboratory reports, and diagnostics reports) related to the event.
- The investigator will then record all relevant AE/SAE/device deficiency information in the participant's medical records, in accordance with the investigator's normal clinical practice and on the appropriate form of the eCRF.
- All ADEs will be entered into the AE eCRF.
- SADEs will be entered into the AE eCRF and submitted to BPV. The site will also complete and submit the study specific paper device report form within 24 hours of site awareness.
- It is not acceptable for the investigator to send photocopies of the participant's medical records to BPV in lieu of completion of the /AE/SAE/device deficiency eCRF page.
- There may be instances when copies of medical records for certain cases are requested by BPV. In this case, all participant identifiers, with the exception of the participant number, will be redacted on the copies of the medical records before submission to BPV.
- The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. Whenever possible, the diagnosis (not the individual signs/symptoms) will be documented as the AE/SAE.
- For device deficiencies, it is very important that the investigator describes any corrective or remedial actions taken to prevent recurrence of the incident.
- A remedial action is any action other than routine maintenance or servicing of a medical device where such action is necessary to prevent recurrence of a device deficiency. This includes any amendment to the device design to prevent recurrence.

Assessment of Intensity

- The investigator will make an assessment of intensity for each AE/SAE/device deficiency reported during the study and assign it to one of the following categories:
  - Mild: An event that is easily tolerated by the participant, causing minimal discomfort and not interfering with everyday activities.
  - Moderate: An event that causes sufficient discomfort and interferes with normal everyday activities.
  - Severe: An event that prevents normal everyday activities. An AE that is assessed as severe should not be confused with a SAE. Severe is a category utilized for rating the intensity of an event; and both AEs and SAEs can be assessed as severe.
- An event is defined as 'serious' when it meets at least 1 of the predefined outcomes as described in the definition of an SAE, NOT when it is rated as severe.

Assessment of Causality

- The investigator is obligated to assess the relationship between study treatment and each occurrence of each AE/SAE/device deficiency.
- A "reasonable possibility" of a relationship conveys that there are facts, evidence, and/or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out.
- The investigator will use clinical judgment to determine the relationship.
- Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to study treatment administration will be considered and investigated.
- The investigator will also consult the IB in his/her assessment.
- For each AE/SAE/device deficiency, the investigator must document in the medical notes that he/she has reviewed the AE/SAE/device deficiency and has provided an assessment of causality.
- There may be situations in which an SAE has occurred and the investigator has minimal information to include in the initial report to BPV. However, it is very important that the investigator always make an assessment of causality for every event before the initial transmission of the SAE data to BPV.
- The investigator may change his/her opinion of causality in light of follow-up information and send a SAE follow-up report with the updated causality assessment.
- The causality assessment is one of the criteria used when determining regulatory reporting requirements.

Follow-up of AE/SAE/device deficiency

- The investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by BPV to elucidate the nature and/or causality of the AE/SAE/device deficiency as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.
- If a participant dies during participation in the study or during a recognized follow-up period, the investigator will provide BPV with a copy of any post mortem findings including histopathology.
- New or updated information will be recorded in the originally completed eCRF.
- The investigator will submit any updated SAE data to BPV within 24 hours of receipt of the information.

10.6.5 Reporting of SAEs

SAE Reporting to BPV via an Electronic Data Collection Tool

- The primary mechanism for reporting an SAE to BPV will be the electronic data collection tool.
- If the electronic system is unavailable, then the site will use the paper SAE data collection tool (see next section) in order to report the event within 24 hours.
- The site will enter the SAE data into the electronic system as soon as it becomes available.
- After the study is completed at a given site, the electronic data collection tool will be taken off-line to prevent the entry of new data or changes to existing data.
- If a site receives a report of a new SAE from a study participant or receives updated data on a previously reported SAE after the electronic data collection tool has been taken off-line, then the site can report this information on a paper SAE form (see next section) or to BPV by telephone.
- Contacts for SAE reporting can be found in Appendix 3, Section 10.3.4.

SAE Reporting to BPV via Paper CRF

- Facsimile transmission of the SAE paper CRF is the preferred method to transmit this information to BPV.
- In rare circumstances and in the absence of facsimile equipment, notification by telephone is acceptable with a copy of the SAE data collection tool sent by overnight mail or courier service.
- Initial notification via telephone does not replace the need for the investigator to complete and sign the SAE CRF pages within the designated reporting time frames.
- Contacts for SAE reporting can be found in Appendix 3, Section 10.3.4.

10.6.6 Reporting of SADEs

SAE Reporting to BPV

NOTE: There are additional reporting obligations for medical device incidents that are potentially related to SAEs that must fulfil the legal responsibility to notify appropriate regulatory authorities and other entities about certain safety information relating to medical devices being used in clinical studies.

- Any device deficiency that is associated with an SAE must be reported to the sponsor within 24 hours after the investigator determines that the event meets the definition of a device deficiency.
- The sponsor shall review all device deficiencies and determine and document in writing whether they could have led to an SAE. These shall be reported to the regulatory authorities and IRBs/IECs as required by national regulations.
- Contacts for SAE reporting can be found in Appendix 3, Section 10.3.4.

10.7 Appendix 7: Abbreviations

Abbreviation	Definition
ACMG	American College of Medical Genetics and Genomics
ADHD	Attention Deficit Hyperactivity Disorder
ADHD RS-IV	Attention Deficit Hyperactivity Disorder Rating Scale
ADL	activities of daily living
AE	adverse event
AESI	adverse events of special interest
ALT	alanine aminotransferase
ANCOVA	Analysis of Covariance
AST	aspartate aminotransferase
AUC	area under the concentration-time curve
BH4	tetrahydrobiopterin
BLA	Biologics License Application
BPV	BioMarin Pharmacovigilance
BRIEF2	Behavior Rating Inventory of Executive Function, Second Edition
C3	complement component 3
C4	complement component 4
CaGIC	Caregiver Global Impression of Change
CaGIS	Caregiver Global Impression of Severity
CGIS	Clinician Global Impression of Severity
CIOMS	Council for International Organizations of Medical Sciences
C_{max}	maximum observed plasma concentration
COA	Clinical Outcome Assessment
CONSORT	Consolidated Standards of Reporting Trials
CRF	case report form
CRP	C-reactive protein
CTCAE	Common Terminology Criteria for Adverse Events
DBL	database lock
DFRF	Directed Follow-up Report Form
DMC	Data Monitoring Committee
DPARP	FDA Division of Pulmonary, Allergy, and Rheumatology Products
DRE	disease-related event
DRI	Dietary Reference Intake
DSM	Diagnostic and Statistical Manual of Mental Disorders
ECG	electrocardiogram

Abbreviation	Definition
EC/IRB	Ethics Committee/Institutional Review Board
eCRF	electronic Case Report Form
EDC	electronic data capture
ET	Early Termination
EU	European Union
FDA	Food and Drug Administration
FSH	follicle stimulating hormone
GCP	good clinical practice
GPP	good publication practice
HAE	hypersensitivity adverse event
HIPAA	Health Insurance Portability and Accountability Act
HPA	hyperphenylalaninemia
HRT	hormonal replacement therapy
HRV	hypersensitivity reaction visit
ICF	Informed Consent Form
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
ICMJE	International Committee of Medical Journal Editors
IgE	immunoglobulin E
IgG	immunoglobulin G
IgM	immunoglobulin M
IMP	investigational medical products
IND	investigational new drug
IP	investigational product
IQ	intelligence quotient
ISR	injection site reaction
I/T/M	Induction/Titration/Maintenance (dosing regimen)
LS	least square
MedDRA	Medical Dictionary for Regulatory Activities
MNT	medical nutritional therapy
NAb	neutralizing antibody
NCI-CTCAE	National Cancer Institute – Common Terminology Criteria for Adverse Events
NIAID/FAAN	National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network
NIMP	non-investigational medical products
NPKUA	National PKU Alliance

Abbreviation	Definition
NSAID	nonsteroidal anti-inflammatory drug
PAH	phenylalanine hydroxylase
PAL	phenylalanine ammonia lyase
PDCO	European Medicines Agency's Paediatric Committee
PEG	polyethylene glycol
PFS	pre-filled syringe
PGIC	Patient Global Impression of Change
PGIS	Patient Global Impression of Severity
Phe	phenylalanine
PIP	Paediatric Investigational Plan
PK	pharmacokinetics
PKU	phenylketonuria
PKU-SSIS	PKU Symptom Severity and Impacts Scale
PRN	pro re nata (as needed)
rAvPAL-PEG	PEGylated recombinant Anabaena variabilis phenylalanine ammonia lyase
RCT	randomized control trial
RDI	recommended daily intake
REMS	Risk Evaluation and Mitigation Strategy
SAE	serious adverse event
SAP	Statistical Analysis Plan
sBLA	supplemental Biologics License Application
SC	Study Completion
SD	standard deviation
SoA	Schedule of Activities
SOI	Statement of Investigator
TAb	total anti-drug antibody
Tyr	tyrosine
ULN	upper limit of normal
US	United States
WOCBP	Woman of Childbearing Potential

10.8 Appendix 8: Protocol Amendment History

The Protocol Amendment Summary of Changes Table for the current amendment is located directly before the Table of Contents (TOC). This is the second amendment to this protocol.

Amendment 1 (ex-US) (06 June 2022):

Overall Rationale for the Amendment

The protocol has been amended following German Federal Institute for Drugs and Medical Devices (BfArM) feedback and for consistency with the Summary of Product Characteristics (SmPC); patients with a history of a severe systemic hypersensitivity reaction or a history of recurrent acute systemic hypersensitivity reactions to a PEGylated medicinal product will be excluded from study participation.

Section Number and Name	Description of Change	Brief Rationale
Section 1.1, Synopsis, and Section 5.2, Exclusion Criteria	Added criterion to exclude patients with a history of a severe systemic hypersensitivity reactions or a history of recurrent acute systemic hypersensitivity reactions to a PEGylated medicinal product from study participation.	The exclusion criterion has been added following German Federal Institute for Drugs and Medical Devices (BfArM) feedback and for consistency with the SmPC.

Amendment 1 (Global) (26 January 2022):

Overall Rationale for the Amendment

The protocol is being amended in response to feedback received from the Food and Drug Administration (FDA) in a request for advice/information letter dated 02 December 2021, and in response to feedback received from the European Medicines Agency's Paediatric Committee (PDCO) in a Paediatric Investigational Plan (PIP) modification positive opinion. Additionally, minor typographic and consistency errors are also being corrected.

Section Number and Name	Description of Change	Brief Rationale
Synopsis Figure 1.2.2 (Dosing Schematic) 4.1 (Overall Design) 5.5 (Study Stopping Criteria) 6.5 (Dose Modification) Table 6.5.1 (Study Drug Dose Titration) 7.1 (Discontinuation of Study Treatment)	Clarified that pegvaliase should be discontinued in participants who have not achieved an adequate response (< 600 µmol/L) after 16 weeks of continuous treatment at 60 mg/day	Response to FDA's advice/information request letter dated 02 December 2021, per US Prescribing Information.

Section Number and Name	Description of Change	Brief Rationale
Synopsis 5.1 (Inclusion Criteria)	Inclusion criterion wording amended to reflect that patients should have failed both sapropterin dihydrochloride and Phe-restricted diet, not one "and/or" the other.	Response to FDA's advice/information request letter dated 02 December 2021
Synopsis 5.1 (Inclusion Criteria) 5.3.1 (Meals and Dietary Restrictions) 8.1.2 (Total Dietary Protein Intake, Dietary Monitoring, and Dietary Protocol Restrictions)	Added "…Inclusion criterion to include the willingness for patients to maintain stable diet under supervision of study dietician or adequately trained designee per investigator during study participation"	As per agreement with PDCO, reflected in the PIP modification approval letter (EMEA-001951-PIP01-16-M02)
Synopsis Table 3.1 (Objectives and Endpoints)	Additional exploratory endpoint added with regards to the comparison of participants' optimal maintenance pegvaliase dose across different age groups within the study	As per agreement with PDCO, reflected in the PIP modification approval letter (EMEA-001951-PIP01-16-M02)
Synopsis Table 1.3.1 (SoA Part 1, Pegvaliase Arm) Table 1.3.2 (SoA Part 2, Pegvaliase Arm) Table 1.3.5 (SoA Part 2, Diet-Only Arm) 4.1 (Overall Design) 4.2 (Scientific Rationale for Study Design) 4.3 (Justification of Dose and Dose Regimen) 6.5 (Dose Modifications)	Clarified dosing administration to occur in a hospital setting for the first 8 weeks and for all subsequent dose escalations.	As per agreement with PDCO, reflected in the PIP modification approval letter (EMEA-001951-PIP01-16-M02)
Synopsis 2.2 (Background) 4.1 (Overall Design) 4.2 (Scientific Rationale for Study Design) 5.1 (Inclusion Criteria)	Added the abbreviation "US" and the eligibility age criterion (12 to 15 years (EU), inclusive) in EU	Clarify the eligibility age criterion of the patient populations for EU and US given the pegvaliase approval in US for 18 years and older and in EU for 16 years and older
5.4 (Screen Failures)	Added clarifying language around participants who are rescreened.	Makes it clear which rescreened participants must performed all Screening Visits procedures and assessments when re-screened.

Section Number and Name	Description of Change	Brief Rationale
8.4 (Pharmacokinetics) Table 1.3.1 (SoA Part 1, Pegvaliase Arm) Table 1.3.2 (SoA Part 2, Pegvaliase Arm) Table 1.3.5 (SoA Part 2, Diet-Only Arm)	Added "A pre-dose sample should be collected within 2 hours of dosing"	PK window for pre-dose is now specified to ensure consistency in the timing of the PK samples for all participants
Synopsis 9.4.2 (Primary Endpoints)	Stratification blood Phe measurement will include 2 blood Phe measurement performed during Screening/Run-In only	The Day 1 pre-dose blood Phe sample for stratification was included in error and has been removed. The two blood Phe samples performed during Screening/Run-In are adequate for treatment stratification.
Table 1.3.1 (SoA Part 1, Pegvaliase Arm) Table 1.3.4 (SoA Part 1, Diet-Only Arm)	Removed "and subject appraisal of metabolic control" from footnotes.	Text had been included in error
Table 1.3.4 (SoA Part 1, Diet-Only Arm)	Tryptase measurement added at Week 73	Was omitted in error
Table 1.3.1 (SoA Part 1, Pegvaliase Arm) Table 1.3.4 (SoA Part 1, Diet-Only Arm)	Column title changed from Study Day -28/1 to Study Day -28/-1	Correcting error of missing minus (–) sign
Table 1.3.1 (SoA Part 1, Pegvaliase Arm) Table 1.3.5 (SoA Part 2, Diet-Only Arm)	Urine pregnancy test added at HRV	For consistency with Table 1.3.2
Table 1.3.1 (SoA Part 1, Pegvaliase Arm)	Complement C3/C4 measurements added at Week 13 and Week 21	For alignment with Table 1.3.4 for Week 85 and Week 96

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