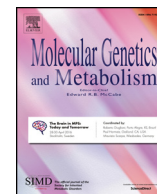




Contents lists available at ScienceDirect

Molecular Genetics and Metabolism

journal homepage: www.elsevier.com/locate/ymgme

Pegvaliase for the treatment of phenylketonuria: Results of a long-term phase 3 clinical trial program (PRISM)

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ARTICLE INFO

Keywords:

Phenylketonuria

Phenylalanine

Recombinant *Anabaena variabilis*

PEGylated phenylalanine ammonia lyase

Pegvaliase

PRISM

ABSTRACT

Background: Phenylketonuria (PKU) is caused by phenylalanine hydroxylase (PAH) deficiency that results in phenylalanine (Phe) accumulation. Pegvaliase, PEGylated recombinant *Anabaena variabilis* phenylalanine ammonia lyase (PAL), converts Phe to trans-cinnamic acid and ammonia, and is a potential enzyme substitution therapy to lower blood Phe in adults with PKU.

Methods: Two Phase 3 studies, PRISM-1 and PRISM-2, evaluated the efficacy and safety of pegvaliase treatment using an induction, titration, and maintenance dosing regimen in adults with PKU. In PRISM-1, pegvaliase-naïve participants with blood Phe > 600 µmol/L were randomized 1:1 to a maintenance dose of 20 mg/day or 40 mg/day of pegvaliase. Participants in PRISM-1 continued pegvaliase treatment in PRISM-2, a 4-part clinical trial that includes an ongoing, open-label, long-term extension study of pegvaliase doses of 5 mg/day to 60 mg/day.

Results: Of 261 participants who received pegvaliase treatment, 72.0% and 32.6% reached ≥12 months and ≥24 months of study treatment, respectively, and 65% are still actively receiving treatment. Mean (SD) blood Phe was 1232.7 (386.4) µmol/L at baseline, 564.5 (531.2) µmol/L at 12 months, and 311.4 (427) µmol/L at 24 months, a decrease from baseline of 51.1% and 68.7%, respectively. Within 24 months, 68.4% of participants achieved blood Phe ≤ 600 µmol/L, 60.7% of participants achieved blood Phe ≤ 360 µmol/L, below the upper limit recommended in the American College of Medical Genetics and Genomics PKU management guidelines, and 51.2% achieved blood Phe ≤ 120 µmol/L, below the upper limit of normal in the unaffected population. Improvements in neuropsychiatric outcomes were associated with reductions in blood Phe and were sustained with long-term pegvaliase treatment. Adverse events (AEs) were more frequent in the first 6 months of exposure (early treatment phase) than after 6 months of exposure (late treatment phase); 99% of AEs were mild or moderate in severity and 96% resolved without dose interruption or reduction. The most common AEs were

Abbreviations: ACMG, American College of Medical Genetics and Genomics; ADHD RS-IV IA, Attention Deficit Hyperactivity Disorder Rating Scale IV inattention subscale; AE, adverse event; CTCAE, Common Terminology Criteria for Adverse Events; DMC, Data Monitoring Committee; HAE, hypersensitivity adverse event; Ig, immunoglobulin; ISR, injection-site reaction; ITT, intent-to-treat; MedDRA, Medical Dictionary for Regulatory Activities; NAb, neutralizing antibodies; NIAID/FAAN, National Institute of Allergy and Infectious Diseases and the Food Allergy and Anaphylaxis Network; PAH, phenylalanine hydroxylase; PAL, phenylalanine ammonia lyase; PEG, polyethylene glycol; Phe, phenylalanine; PKU, phenylketonuria; PKU-POMS, PKU-specific Profile of Mood States; POMS, Profile of Mood States; SD, standard deviation; RDT, randomized discontinuation trial; RDA, recommended dietary allowance; SAE, serious adverse event; TAB, total pegvaliase antibodies

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<https://doi.org/10.1016/j.ymgme.2018.03.006>

Received 14 January 2018; Received in revised form 15 March 2018; Accepted 15 March 2018

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arthralgia (70.5%), injection-site reaction (62.1%), injection-site erythema (47.9%), and headache (47.1%). Acute systemic hypersensitivity events consistent with clinical National Institute of Allergy and Infectious Diseases and the Food Allergy and Anaphylaxis Network anaphylaxis criteria were observed in 12 participants (17 events); of these, 6 participants remained on treatment. Acute systemic hypersensitivity events including potential events of anaphylaxis were not associated with immunoglobulin E, and all events resolved without sequelae.

Conclusion: Results from the PRISM Phase 3 program support the efficacy of pegvaliase for the treatment of adults with PKU, with a manageable safety profile in most participants. The PRISM-2 extension study will continue to assess the long-term effects of pegvaliase treatment.

1. Introduction

Phenylketonuria (PKU; OMIM 261600), an autosomal recessive inborn error of metabolism, is characterized by deficiency of the enzyme phenylalanine hydroxylase (PAH), which metabolizes phenylalanine (Phe) [1]. PAH deficiency leads to elevated blood Phe concentrations, which are toxic to the brain; in adults with PKU, elevated blood Phe is associated with cognitive dysfunction, memory impairment, and behavior and psychiatric problems, such as depression and anxiety, all of which can reduce quality of life [2–4].

Because of the consequences of elevated blood Phe levels, guidelines from the American College of Medical Genetics and Genomics (ACMG) recommend lifelong treatment of PKU, with the primary goal of therapy to lower blood Phe to the range of 120 $\mu\text{mol/L}$ to 360 $\mu\text{mol/L}$ [1]. Treatment involves severe restriction of dietary Phe (found in natural protein foods), supplemented with Phe-free amino acid–modified medical foods and special low-protein foods, alone or with sapropterin dihydrochloride (sapropterin, KUVAN®, BioMarin Pharmaceutical Inc., Novato, CA) [1,5].

Current treatments, however, are ineffective in many adults with PKU due to long-term adherence issues [4,6,7] or inadequate Phe-lowering effects [8–11]. Bik-Multanowski et al. [4] found in a study of 53 adults with PKU, only 10 were able to adhere to Phe restriction for 9 months. The majority of adults with PKU become lost to follow-up for various reasons, and likely have suboptimal metabolic control due to poor adherence to treatment in the absence of support from a metabolic clinic [6,12–14]. Yet even among patients who report dietary Phe restriction, many continue to experience blood Phe concentrations > 1000 $\mu\text{mol/L}$ [9]. In PKU studies, only approximately 20% to 56% of patients in clinical trials of combination sapropterin with dietary Phe restriction responded to treatment [8,10,11], as sapropterin (a cofactor) is only effective in individuals who have residual PAH activity.

Recent publications further highlight the need for novel treatment approaches to help lower elevated blood Phe concentrations [1,14–16]. A 2015 survey of US metabolic clinics with > 1000 actively managed adults with PKU (ie, visited clinic within the last 3 years) estimated that 67% of their patients had blood Phe > 360 $\mu\text{mol/L}$, 45% had blood Phe > 600 $\mu\text{mol/L}$, and 18% had blood Phe > 1200 $\mu\text{mol/L}$ [14]. Similar results were found in a patient self-reporting survey, with only 24% of adults with PKU reporting blood Phe $\leq$ 360 $\mu\text{mol/L}$ within the past year, even though approximately 40% were receiving sapropterin treatment [15].

Pegvaliase, PEGylated recombinant *Anabaena variabilis* phenylalanine ammonia lyase (PAL), is being developed as an enzyme substitution therapy to reduce blood Phe concentration in adults with PKU [17]. Pegvaliase converts Phe to ammonia and trans-cinnamic acid, which are metabolized by the liver and excreted in the urine, respectively [18,19]. PEGylation of the PAL enzyme is associated with a reduced immune response and improved pharmacodynamic stability, which is intended to reduce adverse events (AEs) and increase blood Phe-lowering activity, respectively [17]. Pegvaliase has been investigated in a single Phase 1 clinical trial (NCT00634660) [18], four Phase 2 clinical trials (NCT000924703, NCT01560286, NCT00925054, NCT01212744) [20–22], and two Phase 3 clinical trials, PRISM-1 (NCT01819727) and PRISM-2 (NCT01889862). In PRISM-1, pegvaliase treatment was initiated using a dosing regimen based on the Phase 2 clinical trial experience. Participants in PRISM-1 then enrolled into PRISM-2 to continue pegvaliase treatment and long-term efficacy and safety assessments. Here, we report the combined results of the PRISM-1 and PRISM-2 Phase 3 clinical trials.

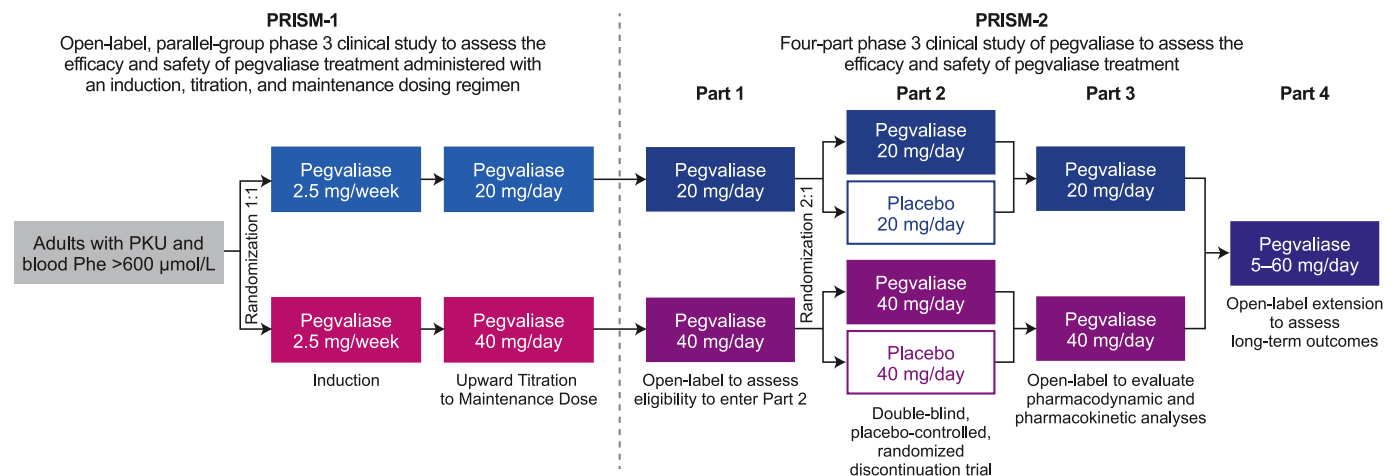


Fig. 1. Phase 3 PRISM Clinical Trial Program Study Design. In PRISM-1, pegvaliase was administered with an induction, titration, and maintenance dosing schedule, in participants randomized 1:1 to titrate to a maintenance dose of 20 mg/day or 40 mg/day. Participants continued pegvaliase treatment in PRISM-2, a subsequent 4-part clinical trial that enrolled participants who were previously exposed to pegvaliase. Part 1 of PRISM-2 identified eligible participants to enter Part 2, a double-blind, randomized discontinuation trial. Participants who completed Part 2 transitioned into Part 3, in which pharmacokinetic and pharmacodynamic assessments were conducted. Participants in PRISM-1 or Parts 1–3 of PRISM-2 ultimately transitioned into Part 4 of PRISM-2, an ongoing, open-label, long-term extension study.

2. Methods

2.1. Study design

Pegvaliase-naïve adults enrolled into PRISM-1, an open-label, multicenter, parallel-group study of pegvaliase administered with an induction, titration, and maintenance dosing schedule, in participants randomized 1:1 to titrate to a maintenance dose of 20 mg/day or 40 mg/day. Participants continued pegvaliase treatment in PRISM-2, a subsequent 4-part clinical study that enrolled participants who were previously exposed to pegvaliase. While 12 participants in Phase 2 studies (165–205 and PAL-003) also enrolled in PRISM-2, the results reported here are limited to participants who initiated pegvaliase treatment in PRISM-1 (Fig. 1).

2.2. Study participants

Adults with PKU aged ≥ 18 years (or aged ≥ 16 years prior to a protocol change in August 2014) were enrolled in PRISM-1 from May 2013 to November 2015 from 31 clinical centers in the United States. Inclusion criteria included a blood Phe concentration of $> 600 \mu\text{mol/L}$ for 6 months prior to enrollment and no previous exposure to pegvaliase. Participants must have discontinued sapropterin (≥ 14 days) and large neutral amino acids (≥ 2 days) prior to the first dose of pegvaliase. For participants receiving neuropsychiatric medications, the dose must have been stable for ≥ 8 weeks prior to enrollment. Additionally, although adherence to a Phe-restricted diet was not required for study inclusion, participants must have been willing and able to maintain consistent dietary protein intake throughout the study.

Participants were excluded if they had used an investigational product or medical device within 30 days prior to screening or had used or planned to use an injectable medication containing polyethylene glycol (PEG) within 3 months prior to screening or during the study. Women who were pregnant or breastfeeding at screening and participants planning to become pregnant (self or partner) during the study were excluded. For sexually active participants, use of contraception was required during and for 4 weeks after study completion. Informed consent was obtained from each participant and the PRISM studies were conducted in accordance with the Declaration of Helsinki.

2.3. Intervention

2.3.1. Pegvaliase dosing regimen

Pegvaliase dosing in PRISM-1 was initiated at 2.5 mg/week for the first 4 weeks. Dose and dose frequency were then gradually increased in the titration period to the randomized maintenance dose of 20 mg/day or 40 mg/day. Any participants unable to titrate or maintain a randomized 20 mg/day or 40 mg/day dose of pegvaliase in PRISM-1 enrolled directly into PRISM-2 Part 4, an open-label extension, where maintenance dose could be adjusted based on individual efficacy and tolerability. Participants who reached the maintenance dose in PRISM-1 continued the same dose in Part 1 of PRISM-2. In Part 2, participants were randomized to either continue their dose of pegvaliase (20 mg/day or 40 mg/day) or placebo. In Part 3, participants received 20 mg/day or 40 mg/day pegvaliase (as per randomization in PRISM-1). Per protocol in Part 4, pegvaliase dose was increased to 40 mg/day, unless not tolerated. Dose increases up to 60 mg/day were based on investigator and medical monitor discretion for participants who had received ≥ 52 weeks of pegvaliase treatment, including ≥ 8 weeks of pegvaliase dosed at 40 mg/day. In Part 4, the dose of pegvaliase could be adjusted between 5 mg/day and 60 mg/day, based on investigator-determined efficacy and tolerability (Supplementary Table 1).

2.3.2. Pegvaliase administration

Pegvaliase was provided for subcutaneous self-administration in a vial with syringes until pre-filled syringes were developed in 2014.

Initial injections were performed in the clinic and participants were required to demonstrate competency in self-administration prior to continuing self-administration at home. Pegvaliase was administered at the same time each day with alternating subcutaneous sites (eg, back of upper arm, front of thigh, abdomen, buttocks) for each injection and at least one-inch separation between injection sites in cases of multiple injections (eg, two 20 mg injections given for a 40 mg dose). Participants recorded the date, time, volume, and injection location of all pegvaliase doses.

2.4. Mitigation of hypersensitivity adverse events

At the initiation of PRISM-1 and PRISM-2, reduction or interruption of dosing was allowed for participants with AEs of at least moderate severity (Common Terminology Criteria for Adverse Events [CTCAE] Grade 2) and premedication to reduce the severity of hypersensitivity adverse events (HAEs) was also allowed based on investigator discretion. During the study (protocol change in August 2014), additional safety procedures were implemented to minimize the risk of acute systemic hypersensitivity events. Investigators were given greater flexibility to reduce or interrupt pegvaliase dosing due to mild AEs (CTCAE Grade 1). Premedication to reduce the severity of HAEs, which was previously elective, was required prior to each pegvaliase dose during the induction and titration periods; the premedication regimen consisted of a histamine H1 receptor antagonist, an H2 receptor antagonist, and, if tolerated, an antipyretic. An observer was required to be present during pegvaliase administration for the first 16 weeks of treatment. Participants also received additional education on identifying and responding to HAE signs and symptoms and were given epinephrine injection pens for use in the event of an acute systemic hypersensitivity event, including potential events of anaphylaxis. Sensitivity analyses evaluating early pegvaliase treatment discontinuation and adverse events before or after implementation of additional safety procedures are described in Section 3.1 and provided in the supplement.

2.5. Dietary protein intake and supplementation

Participants recorded all dietary protein intake from medical food and natural food for 3 consecutive days prior to each clinic visit in diet diaries, which were analyzed by dietitians using MetabolicPro® (Genetic Metabolic Dietitians International, Decatur, Georgia, USA). Participants were counseled by dietitians to maintain consistent protein intake from natural food and medical food, within 10% of baseline, and instructed to take 500 mg of supplemental tyrosine 3 times per day with meals. If blood Phe levels decreased to $\leq 30 \mu\text{mol/L}$, participants were instructed to increase their intake of natural protein and/or decrease medical protein: Participants with natural protein intake less than the recommended dietary allowance (RDA) were instructed to increase natural protein intake by 10 g/day and decrease medical food protein intake by 5 g/day. Participants with natural protein intake more than the RDA were instructed to increase natural protein intake by 10% and decrease medical food protein by 5 g/day, unless natural protein intake was > 2 times the RDA, in which participants were instructed to maintain their current dietary protein intake.

2.6. Assessments

Blood Phe and safety assessments were conducted at baseline, weekly during the induction period, and at least monthly during the titration and maintenance periods until Week 25 of PRISM-2 Part 4, when assessments were conducted bimonthly. Neuropsychiatric assessments were conducted at baseline and at the termination visit in PRISM-1 and at least bimonthly during PRISM-2. Immunogenicity testing was performed at baseline, Week 3, Week 4, and at least bimonthly thereafter.

2.6.1. Neuropsychiatric assessments

Additional secondary efficacy measures were added to the ongoing PRISM-1 and PRISM-2 clinical trials (protocol change in January 2014) to evaluate change in attention and mood symptoms, measured by the inattention subscale of the Attention Deficit Hyperactivity Disorder Rating Scale IV (ADHD RS-IV IA) and the Profile of Mood States (POMS) scale. To reduce variability between sites, designated experienced raters were trained at each site in administering the assessment tools, which have been validated for use in PKU [24,25].

The investigator-rated ADHD RS-IV IA subscale is a 9-item questionnaire with scores ranging from 0 to 27, with higher scores indicating a greater degree of impairment and a score > 9 defined in the study as indicating the presence of inattention symptoms [25]. The inattention subscale of the ADHD RS-IV was used because it is considered more relevant than the hyperactivity subscale for patients with PKU, as inattention symptoms are more prevalent in adults with PKU [26,27].

The POMS consists of a 65-item questionnaire rated using a 5-point Likert scale, and the sum of the scores is reported; scores range from -32 to 200 , with higher scores indicating greater mood symptoms. The PKU-specific POMS (PKU-POMS) is a subset of the POMS instrument that was developed for the PRISM studies to assess transient and variable mood states relevant to PKU [24]. It consists of a 20-item questionnaire and is rated using the same 5-point Likert scale used in the POMS, with a score range of -12 to 58 . A 3-item confusion subscale score of the PKU-POMS, which ranges from 0 to 11 , was also included, based on interim analysis of PRISM-1 data suggesting this symptom was the most sensitive to changes in blood Phe. PKU-POMS and the PKU-POMS confusion subscale scores reported here were derived from results of the POMS questionnaire, which was self-administered by study participants.

2.6.2. Safety endpoints

Safety was monitored by assessment of vital signs, physical examination, electrocardiograms, AEs (coded by preferred terms using Medical Dictionary for Regulatory Activities [MedDRA] version 18.0) [28], and clinical laboratory tests (chemistry, hematology, and urinalysis).

AEs of special interest for purposes of safety monitoring and analysis included all HAEs, injection-site reaction (ISR), injection-site skin reaction lasting ≥ 14 days, generalized skin reaction lasting ≥ 14 days, arthralgia, and acute systemic hypersensitivity events. HAEs were identified using a modified hypersensitivity standard MedDRA query (SMQ), which included additional preferred term AEs of arthralgia, arthritis, eye inflammation, eye irritation, eye pain, joint stiffness, joint swelling, pyrexia, blurred vision and polyarthritides, and the broad algorithmic anaphylactic reaction SMQ. Injection-site reactions were identified using the MedDRA high-level term of injection-site reaction. Injection-site skin reactions were identified using a specified list of MedDRA preferred terms with a reported duration of ≥ 14 days. Generalized skin reactions were identified using a specified list of MedDRA preferred terms and any events identified by the MedDRA broad vasculitis SMQ, with a reported duration lasting ≥ 14 days.

Potential acute systemic hypersensitivity events were identified using the hypersensitivity SMQ, anaphylactic reaction SMQ, and MedDRA preferred terms to identify all reported preferred terms that could potentially be component manifestations of acute systemic hypersensitivity events, as well as all AEs reported as anaphylactic reactions by preferred term. All potential acute systemic hypersensitivity events were reviewed in an adjudication by an allergist/immunologist independent of the clinical site and sponsor, to identify events consistent with clinical criteria of anaphylaxis defined by the National Institute of Allergy and Infectious Diseases/Food Allergy and Anaphylaxis Network (NIAID/FAAN) [29] and Brown's severe criteria (ie, hypoxia, hypotension, or neurologic compromise) [30].

An ad-hoc analysis evaluating patient discontinuation before and

after implementation of additional safety procedures during early treatment was also conducted.

In addition to monitoring and oversight by the sponsor, a Data Monitoring Committee (DMC) was established for the study duration. The DMC was an independent committee that monitored the safety of participants and acted in an advisory capacity to the sponsor.

2.6.3. Immunogenicity

Validated assays designed for the PRISM studies assessed anti-drug antibody titer levels for total anti-pegvaliase antibodies (TAb), PAL immunoglobulin (Ig) M, PEG IgM, PAL IgG, PEG IgG, and neutralizing antibodies (NAb). In the event of an HAE that required a clinic visit, sampling and testing were performed for pegvaliase-specific IgE after immunodepletion of IgG and IgM from the samples.

2.7. Statistical analysis

The primary intent-to-treat (ITT) population for efficacy, safety, and immunogenicity analyses consisted of all participants who initiated pegvaliase treatment in PRISM-1, and included all data collected in PRISM-1 and PRISM-2 for these participants as of September 23, 2016. Descriptive summaries of continuous variables included the number of participants (n), mean, standard deviation (SD), median, minimum, and maximum. Descriptive summaries of categorical variables included n and percent. The baseline value of an assessment was defined as the last available measurement prior to the first administration of pegvaliase in PRISM-1 (ie, pegvaliase-naïve baseline). Due to the variable nature of visit schedules across different study parts, by-visit summaries at nominal study visits could not be produced. As a result, blood Phe data were summarized at monthly intervals relative to baseline. Neuropsychiatric endpoints were summarized at 3-month intervals relative to baseline due to less frequent data collection.

A summary of efficacy endpoints over time included: observed and percent change values from baseline in blood Phe concentration, ADHD RS-IV IA subscale score, POMS score, PKU-POMS score, and PKU-POMS confusion subscale score. Kaplan-Meier estimate analyses of time to blood Phe $\leq 600 \mu\text{mol/L}$, $\leq 360 \mu\text{mol/L}$, and $\leq 120 \mu\text{mol/L}$ were also performed, providing a cumulative estimate for achievement of blood Phe threshold during at least one assessment within the specified time period. Among participants who did not achieve the specified blood Phe threshold, time to reduction was censored at their last blood Phe assessment date, with the exception of those who discontinued pegvaliase and/or the study due to an adverse event, physician's decision, or withdrawal by participant, for whom the Phe reduction target was considered unachievable. Additional analyses were performed to assess the relationship between the change in blood Phe concentration and the change in ADHD RS-IV IA subscale scores, reported as the change from baseline to last observation in ADHD RS-IV IA score over time and by quartile of change in blood Phe from baseline, for all participants and for those with baseline ADHD RS-IV IA scores > 9 (ie, inattention subgroup).

For the safety analyses, data on the incidence, exposure-adjusted event rate, severity based on CTCAE grade (Grade 1, mild; Grade 2, moderate; Grade 3, severe but not immediately life-threatening; Grade 4, life-threatening) [31], and relationship to pegvaliase of all treatment-emergent AEs were compared between the early treatment phase (first 6 months of treatment), the late treatment phase (after first 6 months of treatment), and for the entire study duration.

The exposure-adjusted event rate analysis, reported as events per person-year, was performed to control for differences in duration of study participation that allowed for an exposure-adjusted comparison of AE frequency. The event rate comparisons between the early (< 6 months) and late treatment (> 6 months) phases were comparisons between different study populations (ie, participants who discontinued the study during the first 6 months were not included in the 6- to 12-month safety analysis). Sensitivity analyses were also

performed to assess for consistency of trends by evaluating the impact of early dropout on the assessment of blood Phe concentration. For participants who terminated treatment early, the last observed record was carried forward to impute the record at monthly intervals.

3. Results

3.1. Participant disposition and baseline characteristics

The demographic characteristics of enrolled patients are reported in Table 1. Of the 261 participants in PRISM-1, 131 participants were randomized to the 20 mg/day pegvaliase arm and 130 participants to the 40 mg/day pegvaliase arm in PRISM-1. The mean (SD) participant age at enrollment was 29.2 (8.8) years, and the study population was evenly split between females and males. In PRISM-1, the mean (SD) time to titrate up to the maintenance dose was 11.5 (3.0) weeks for participants in the 20 mg/day dose group ($n = 103$) and 14.0 (4.7) weeks for participants in the 40 mg/day dose group ($n = 92$). Of the participants who reached maintenance dosing in PRISM-1, 95% reached maintenance dosing by 18.1 weeks and 23.9 weeks in the 20 mg/day and 40 mg/day dose groups, respectively.

After PRISM-1, 203 (77.8%) participants continued to PRISM-2 (Fig. 2). Of the 58 participants who did not enter PRISM-2, 54 discontinued pegvaliase early because of AEs ($n = 29$), withdrawal by participant ($n = 14$), physician decision ($n = 4$), pregnancy ($n = 2$), protocol deviation ($n = 2$), lost to follow-up ($n = 1$), or other reason ($n = 2$) and 4 participants chose not to continue pegvaliase dosing in PRISM-2. Of the 203 participants entering PRISM-2, 51 enrolled directly into Part 4 from PRISM-1 (either due to early closure of PRISM-1 after PRISM-2 Part 2 enrollment closed [$n = 35$] or because they were unable to reach the randomized dose in PRISM-1 [$n = 16$]). A total of 139 subjects entered Part 4 after participating in Parts 1, 2, or 3 of PRISM-2: 54 entered Part 4 after Part 1 due to Part 2 enrollment closure ($n = 10$), ineligibility for Part 2 ($n = 39$), or other reasons ($n = 5$); 3 entered Part 4 after Part 2; and all 82 subjects in Part 3 entered Part 4.

A total of 88 participants (33.7%) discontinued pegvaliase during the PRISM-1 and PRISM-2 studies. The most common reason for discontinuation of pegvaliase was an AE ($n = 40$, 15.3%), as further described in Section 3.6. Other reasons were withdrawal by participant ($n = 24$, 9.2%), physician decision ($n = 8$, 3.1%), lost to follow-up ($n = 7$, 2.7%), other ($n = 4$, 1.5%), protocol deviation ($n = 3$, 1.1%), and pregnancy ($n = 2$, 0.8%). Of the 173 participants remaining in the long-term extension study, 4 had discontinued pegvaliase but remained in the study for ongoing assessment.

After implementation of additional safety procedures, early pegvaliase treatment discontinuation within the first 6 months of treatment, particularly due to AEs, decreased from 15.4% (22/143) to 5.9% (7/118) (Supplementary Table 2).

3.2. Pegvaliase exposure

At the time of the datacut, participants received pegvaliase treatment for a mean of 18.5 months. Of the 261 participants who enrolled and received pegvaliase treatment, 72.0% and 32.6% reached ≥ 12 months and ≥ 24 months of study treatment, respectively, and 65% are still actively receiving treatment. Of the 261 participants, 194 had at least 80% compliance with pegvaliase treatment, with 46% of participants receiving between 40 mg/day and 60 mg/day as the most recent dose in the study (Table 2).

3.3. Protein intake

Total daily protein intake remained relatively stable from a mean (SD) of 64.8 (32.2) g at baseline to 71.6 (24.3) g at 12 months and 77.4 (20.8) g at 24 months (Supplementary Table 3). Protein intake from medical food decreased, from 26.3 (28.5) g at baseline to 24.2 (26.0) g

at 12 months and 18.4 (25.2) g at 24 months. Dietary Phe intake increased, from a mean (SD) of 1700.2 (1194.4) mg at baseline to 2123.2 (1302.2) mg at 12 months and 2679.7 (1285.7) mg at 24 months.

3.4. Blood Phe concentration

The decline in mean (SD) blood Phe concentration from a baseline of 1232.7 $\mu\text{mol/L}$ (386.36) was sustained through 24 months of follow-up (Fig. 3). At 12 months, the mean (SD) blood Phe concentration was 564.5 $\mu\text{mol/L}$ (531.2), a 51.1% decrease from baseline ($n = 164$); at 24 months, the mean (SD) blood Phe concentration was 311.4 $\mu\text{mol/L}$ (426.6), a 68.7% decrease from baseline ($n = 51$). Similar trends were observed in a sensitivity analysis evaluating the change in blood Phe in participants completing at least one year of treatment.

A Kaplan-Meier analysis estimated that 71.8% (95% CI: 66.2%, 77.2%) of participants achieved a $\geq 20\%$ reduction in blood Phe (an

Table 1

Treatment-naïve baseline and demographics characteristics (intent-to-treat population, $N = 261$).

	All participants ($N = 261$)
Age at enrollment	
Mean (SD), years	29.2 (8.8)
Sex	
Female, n (%)	130 (49.8%)
Race	
White, n (%)	254 (97.3%)
Ethnicity	
Not Hispanic or Latino, n (%)	253 (96.9%)
Height	$n = 260$
Mean (SD), cm	168.1 (9.5)
Body mass index	$n = 260$
Mean (SD), kg/m^2	28.4 (6.7)
Total protein intake	$n = 250$
Mean (SD), g/day	64.89 (32.2)
Median, g/day	62.6
Protein intake from natural food	$n = 250$
Mean (SD), g/day	38.5 (27.7)
Median, g/day	29.9
Protein intake from medical food	$n = 250$
Mean (SD), g/day	26.3 (28.5)
Median, g/day	16.8
Receiving protein from medical food, n (%)	149 (57.1%)
> 75% of protein intake from medical food, n (%)	41 (15.7%)
Dietary Phe intake	$n = 250$
Mean (SD), mg/day	1700.2 (1194.4)
Median, mg/day	1357.0
Blood Phe	$n = 261$
Mean (SD), $\mu\text{mol/L}$	1232.7 (386.4)
Median, $\mu\text{mol/L}$	1221.0
Neurocognitive/neuropsychiatric measures	
ADHD RS-IV total score	$n = 169$
Mean (SD)	15.8 (10.13)
ADHD RS-IV inattention subscale scores	$n = 253$
Mean (SD)	9.8 (6.12)
ADHD RS-IV inattention subscale score > 9	$n = 116$
Mean (SD)	15.3 (4.06)
POMS score	$n = 170$
Mean (SD)	35.7 (30.66)
PKU-POMS score	$n = 170$
Mean (SD)	15.9 (13.21)
PKU-POMS confusion subscale score	$n = 170$
Mean (SD)	4.0 (2.67)

ADHD RS-IV, Attention Deficit Hyperactivity Disorder Rating Scale IV; Phe, phenylalanine; PKU, phenylketonuria; POMS, Profile of Mood States; SD, standard deviation. Baseline was defined as the last measurement before the first dose of pegvaliase (i.e., treatment-naïve) in PRISM-1. Total protein intake includes medical food and natural protein dietary intakes and was calculated as the daily average intake over 3 days prior to the assessment point. Protein and Phe intakes were calculated as the daily average intake over 3 days prior to the assessment point. Sample size indicated if data was not available for all subjects.

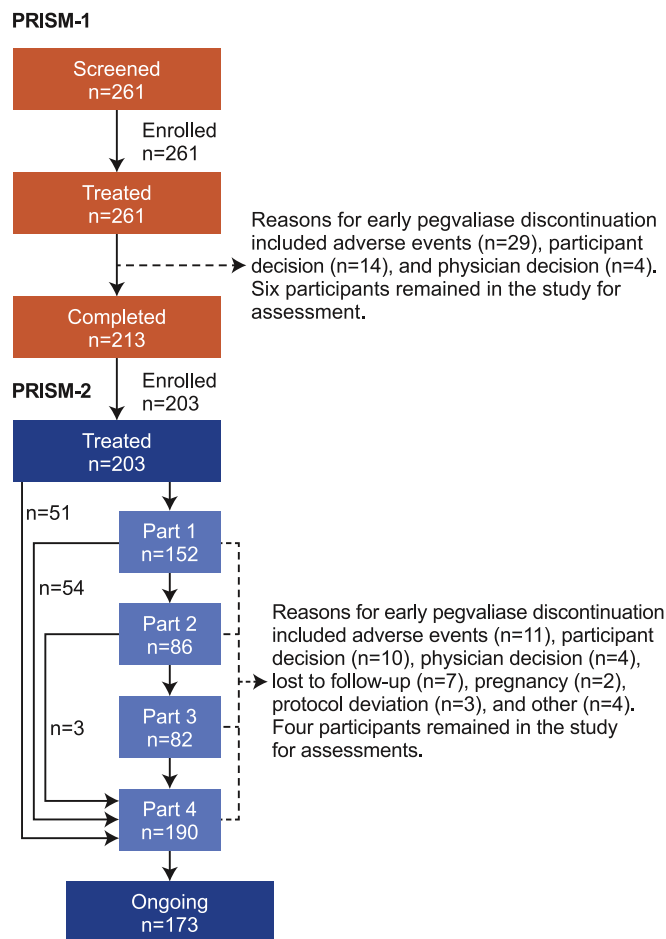


Fig. 2. Phase 3 PRISM clinical trial program participant disposition. PRISM-1 enrolled 261 participants with PKU to receive pegvaliase administered with an introduction, titration, and maintenance dosing schedule. PRISM-2, a 4-part clinical trial, enrolled 203 participants from PRISM-1. Participants ultimately transitioned into Part 4 of PRISM-2, an ongoing, open-label, long-term extension study after completing PRISM-1 or Parts 1, 2, or 3 of PRISM-2.

early signal of pegvaliase pharmacologic effect) from treatment-naïve baseline by 12 months and 78.3% (95% CI: 73.0%, 83.3%) by 24 months. Blood Phe reduction to ≤ 360 $\mu\text{mol/L}$ was achieved by 44.0% (95% CI: 38.2%, 50.4%) of participants by 12 months and 60.7% (95% CI: 54.4%, 67.1%) by 24 months, and 51.2% (95% CI, 44.8%, 58.0%) of participants achieved a blood Phe concentration of ≤ 120 $\mu\text{mol/L}$ by 24 months (Fig. 4).

3.5. Inattention and mood symptoms

ADHD RS-IV IA subscale scores showed declines that were maintained with long-term pegvaliase treatment, suggesting improvement in inattention symptoms. After 12 months ($n = 178$), scores were reduced to a mean (SD) of 5.0 (4.9), a 4.7-point (5.6) decline from the baseline score of 9.8 (6.1) ($n = 253$). This decline was maintained at 24 months of pegvaliase dosing ($n = 89$), with a mean (SD) score of 4.5 (4.7), a 6.4-point (5.9) decline from baseline.

Reduction in the ADHD RS-IV IA subscale scores had a temporal association with reduction in blood Phe. Analysis of the change from baseline in ADHD RS-IV IA subscale score and blood Phe at last observation demonstrated that the 53 participants in the quartile with greatest blood Phe reduction ($-2143, 1134.5$) experienced the greatest reduction in ADHD RS-IV IA subscale score, a mean (SD) decrease of 7.5 (5.6) points (Supplementary Figs. 1 and 2).

The magnitude of the change from baseline in ADHD RS-IV IA

Table 2

Pegvaliase exposure (intent-to-treat population, $N = 261$).

	All participants ($N = 261$)
Duration of pegvaliase treatment, months ^a	
Mean (SD)	18.5 (11.6)
Median	16.3
Duration of pegvaliase treatment, n (%)	
≥ 6 months	208 (79.7%)
≥ 12 months	188 (72.0%)
≥ 24 months	85 (32.6%)
≥ 36 months	20 (7.7%)
Treatment adherence rate, % ^b	
Mean (SD)	81.4 (21.3)
Median	89.3
Treatment adherence rate $\geq 80\%$, n (%) ^b	194 (74.3%)
Last daily dose received, n (%) ^c	
< 20 mg/day	49 (18.8%)
≥ 20 to < 40 mg/day	56 (21.5%)
≥ 40 to < 60 mg/day	120 (46.0%)
≥ 60 mg/day	36 (13.8%)

SD, standard deviation.

^a Time from the first dose to the last dose administered across all studies in which a participant was enrolled. Intervals of missing doses that were > 28 consecutive days were excluded from the calculation of treatment duration.

^b Calculated as $100 \times (\text{actual drug taken per participant} / \text{total drug dispensed})$.

^c Last dose administered as of the data cut-off on September 23, 2016.

subscale scores was greater for those participants with baseline ADHD RS-IV IA subscale scores > 9 and was maintained at 24 months of pegvaliase dosing ($n = 45$), with a mean (SD) score of 6.3 (4.6), a 9.6-point (5.8) decline from baseline score of 15.3 (4.1) ($n = 116$) (Figs. 5 and 6).

Similar trends were observed with mood scores, which also improved over time in parallel with blood Phe reductions. The mean (SD) POMS score decreased from 35.7 (30.7) at baseline ($n = 170$) to 22.1 (29.9) at 12 months ($n = 181$) and 18.3 (29.6) at 24 months ($n = 90$). Similarly, the mean (SD) PKU-POMS score decreased from 15.9 (13.3) at baseline ($n = 170$) to 8.5 (12.5) at 12 months ($n = 181$) and 6.6 (12.6) at 24 months ($n = 90$), as did the PKU-POMS confusion subscale score, which decreased from 4.0 (2.7) at baseline ($n = 170$) to 2.4 (2.1) at 12 months ($n = 181$) and 2.0 (2.2) at 24 months ($n = 90$).

3.6. Safety

All 261 participants reported at least 1 AE during the study that was assessed by the investigator to be related to study drug. Most AEs were mild or moderate (99%) and resolved without dose change or interruption (96%). The most commonly reported AEs by preferred term were arthralgia (70.5% of patients), ISR (62.1%), injection-site erythema (47.9%), and headache (47.1%), with exposure-adjusted rates of 2.6, 4.3, 1.7, and 1.6 events per person-year, respectively (Supplementary Table 4). The most common AEs leading to discontinuation of pegvaliase by preferred term were anaphylactic reaction (2.7% of participants, 4 of these 7 participants had a confirmed acute systemic hypersensitivity event, described below), arthralgia (2.7%, $n = 7$), ISR (1.1%, $n = 3$), and generalized rash (0.8%, $n = 2$).

Thirty-four of 61 serious adverse events (SAEs) in 47 participants were assessed by investigators as treatment-related. There was one death in the study, a work-related accidental electrocution that was not related to the study drug. The exposure-adjusted rate for SAEs was 0.15 events per person-year. The most common SAEs by preferred term were anaphylactic reaction (3.8% of participants, 7 of 10 events were confirmed acute systemic hypersensitivity events) and hypersensitivity (2.3%), with exposure-adjusted rates of 0.02 and 0.01 events per person-year, respectively.

Seventeen acute systemic hypersensitivity events, confirmed by an

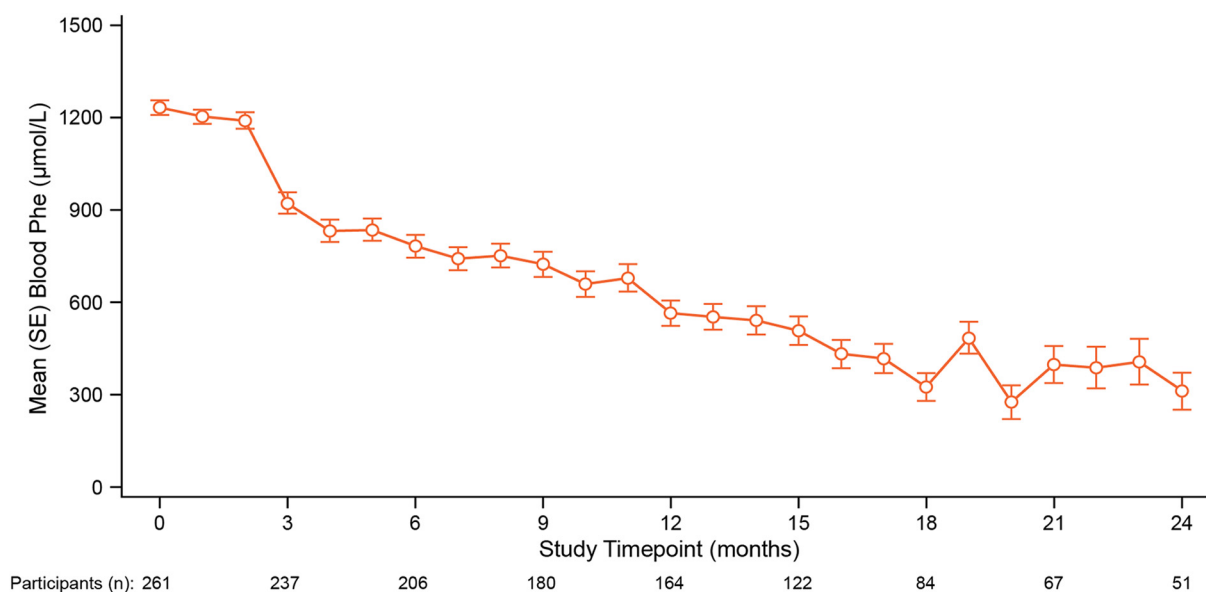


Fig. 3. Mean blood Phe concentration over time (intent-to-treat population; $N = 261$). Sample size reflects participants with data available at study timepoint and who have reached study timepoint at data cut; study is ongoing. Error bars represent standard error. SE, standard error.

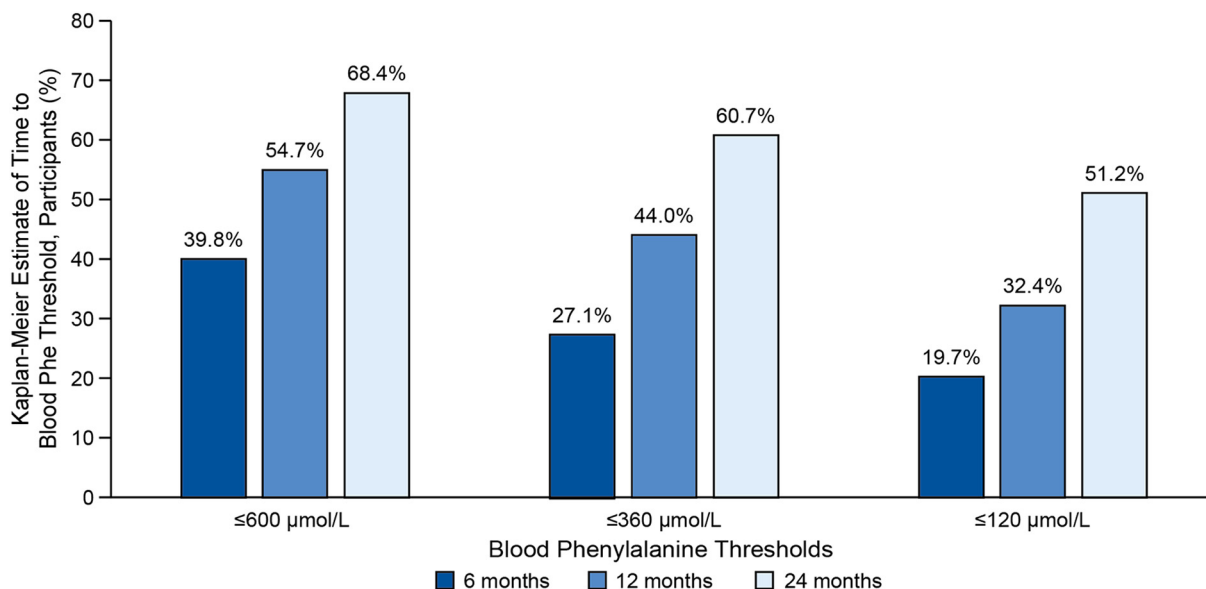


Fig. 4. Kaplan-Meier analyses of time to blood Phe thresholds (intent-to-treat population; $N = 261$). Percentages indicate cumulative estimate for achievement of blood Phe threshold during at least one assessment within the specified time period. Among participants who did not achieve the specified blood Phe threshold, time to reduction was censored at their last blood Phe assessment date, with the exception of those who discontinued pegvaliase and/or the study due to an adverse event, physician's decision, or withdrawal by participant, for whom the Phe reduction target was considered unachievable.

independent allergist/immunologist, occurred in 12 participants (4.6%). Two of these events met Brown's severe criteria based on hypotension ($n = 1$) or hypoxia ($n = 1$) experienced during the event. Epinephrine was administered in 6 events, and no events required vasopressors or intubation. Five events were self-limiting and received no intervention (treatment or dose change). Events occurred within a median of 1.8 min after pegvaliase administration. All events were of short duration and resolved without sequelae. All of the events occurred after the first 50 days of dosing. None of the participants who experienced an event were confirmed positive for drug-specific IgE at or near the time of the event. Six participants discontinued the study after an acute systemic hypersensitivity event, and the remaining 6 participants continued dosing. Of the 6 participants who continued dosing, 2 had additional events that did not occur directly after restarting pegvaliase;

all 6 participants remained on treatment at the time of the data-cut. The incidence of acute systemic hypersensitivity events was higher in the first year compared to the period after the first year. The rate of Brown's severe events in early treatment (< 6 months) decreased from 0.04 events per person-year to 0 events after implementing additional safety procedures as described in methods.

HAEs were reported in 93.5% of participants, with an overall exposure-adjusted rate of 7.5 events per person-year. Almost all HAEs were mild or moderate in severity (98.7%), resolved in < 14 days (84.9%) and resolved without dose change (91.4%). The most frequent HAE-related events by preferred term were arthralgia (70.5% of participants), rash (34.5%), and pruritus (27.6%).

Injection site-related AEs occurred in 92% of participants; most events were mild or moderate in severity (99.9%) with 92.3% resolving

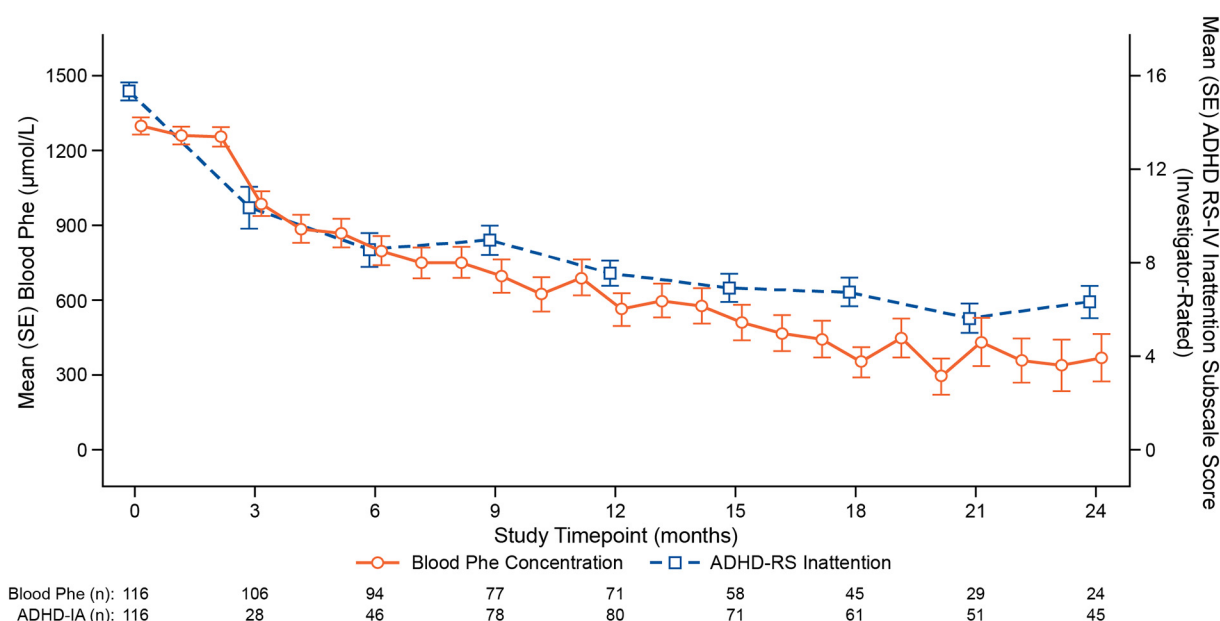


Fig. 5. Blood Phe concentration and investigator-rated ADHD RS-IV IA scores in participants with baseline scores > 9 (inattention subgroup, $N = 116$). Sample size reflects participants with data available at study timepoint and who have reached study timepoint at data cut; study is ongoing. ADHD RS-IV IA, Attention Deficit Hyperactivity Disorder Rating Scale IV inattention subscale; SE, standard error.

in < 14 days and 99% of events resolving without dose change. The most frequent injection site-related events by preferred term were ISR (62.1% of participants), injection-site erythema (47.9%), and injection-site bruising (32.2%).

All arthralgia events were mild or moderate in severity and 79.6% of events resolved in < 14 days. Most arthralgia events did not lead to a dose change (87.7% of events); pegvaliase dose was interrupted or reduced for 9.9% of events and withdrawn for < 1% of events.

Overall, AEs were reported more frequently in the early treatment phase (≤ 6 months) and AEs decreased in frequency with prolonged pegvaliase exposure. Sensitivity analyses evaluating adverse events before and after implementation of additional safety procedures demonstrate similar trends (Supplementary Table 5). The event rate per person-year was 58.6 in the early treatment phase and declined to 19.4

in the late treatment phase (Table 3).

No events suggesting immune complex-mediated end-organ damage, including renal failure, hemolytic anemia, events suggestive of serositis (such as peritonitis, pericarditis, and pleuritis), central nervous system manifestations (such as cerebrovascular accidents or transient ischemic attacks), or myocardial ischemic events related to pegvaliase were observed.

3.7. Immunogenicity

Participants developed a sustained mean TAb titer response. Participants also developed a sustained anti-PAL antibody response and a transient anti-PEG antibody response. Mean PAL IgM and PAL IgG titers peaked approximately 3 months after initiation of pegvaliase,

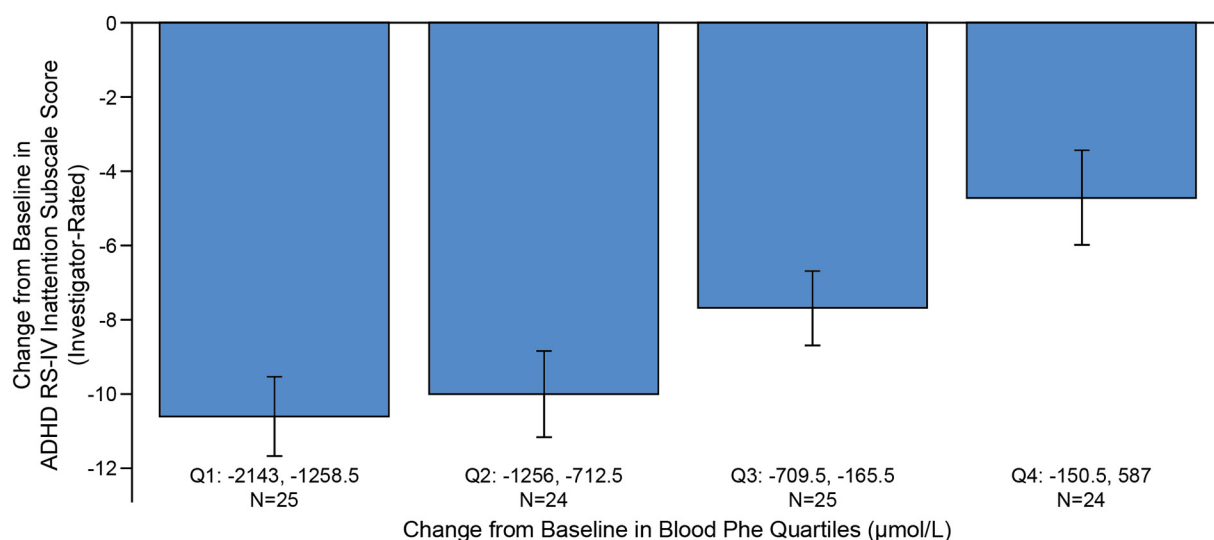


Fig. 6. Change from baseline in ADHD RS-IV IA subscore by change from baseline in blood Phe quartiles at last observation for the subgroup with baseline scores > 9 (inattention subgroup, $N = 98$). Sample size reflects participants with data available at baseline and at least one follow-up measure by data cut; study is ongoing. Blood Phe measurement used the average of the last 2 blood Phe measurements on or before the last ADHD RS-IV IA score collection date. ADHD-RS, Attention Deficit Hyperactivity Disorder Rating Scale IV inattention subscale.

Table 3Adverse event overview by treatment phase, reported as event rate (events/person-years) and total number of events (intent-to-treat population, $N = 261$).

	Early treatment phase (≤ 6 months)	Late treatment phase (> 6 months)				Total	Overall
		> 6 to ≤ 12 months	> 12 to ≤ 24 months	> 24 to ≤ 36 months	> 36 months		
Participants, N	$N = 261$	$N = 210$	$N = 188$	$N = 88$	$N = 23$	$N = 210$	$N = 261$
Total treatment exposure, person-years ^a	115.6	96.8	125.3	59.8	3.5	285.5	401.3
Event rate, per person-year (total number of events)							
AEs	58.6 (6774)	23.7 (2294)	19.1 (2394)	13.4 (802)	17.6 (61)	19.4 (5551)	30.7 (12325)
SAEs	0.2 (26)	0.2 (20)	0.1 (13)	0.03 (2)	0	0.1 (35)	0.2 (61)
HAEs	15.6 (1805)	4.3 (411)	4.9 (610)	2.0 (120)	1.2 (4)	4.0 (1145)	7.4 (2950)
Acute systemic hypersensitivity events	0.05 (6)	0.09 (9)	0.01 (1)	0.02 (1)	0	0.04 (11)	0.04 (17)
Injection-site reactions	25.5 (2953)	6.2 (595)	3.4 (428)	2.1 (124)	4.9 (17)	4.1 (1164)	10.3 (4117)
Injection-site skin reactions lasting ≥ 14 days	1.3 (153)	0.7 (66)	0.6 (75)	0.3 (20)	0	0.6 (161)	0.8 (314)
Generalized skin reactions lasting ≥ 14 days	0.8 (93)	0.3 (32)	0.3 (36)	0.2 (10)	0.3 (1)	0.3 (79)	0.4 (172)
Arthralgia	6.9 (794)	1.5 (147)	0.7 (90)	0.3 (20)	0.3 (1)	0.9 (258)	2.6 (1052)

AE, adverse event; HAE, hypersensitivity AE; SAE, serious AE. For each treatment phase and time interval, only AEs with onset within that phase or interval were included. Event rate was calculated as total number of events divided by person-years of exposure.

^a Total treatment exposure was the aggregated duration of treatment across all participants (for each participant, time from the first dose to 30 days after the last dose administered across all studies in which the participant was enrolled). Intervals of missing doses that were > 28 consecutive days were excluded from the calculation of treatment duration.

then remained stable with long-term treatment (Fig. 7). Mean PEG IgM and PEG IgG titers peaked 1 to 3 months after treatment initiation. Mean PEG IgM titers then returned to baseline levels by 9 months of treatment, with low titer levels of PEG IgM still detectable at the last timepoint of 24 months. Mean PEG IgG titers returned to baseline level by 9 months of treatment and remained near baseline level or were undetectable beyond that timepoint. The mean NAb titer response developed by month 3 remained stable with long-term treatment, as mean blood Phe levels were decreasing. Drug-specific IgE was not detected at or near the time of any acute systemic hypersensitivity event, including potential events of anaphylaxis. Mean complement component 4 (C4) levels decreased and remained within the normal range. Mean complement component 3 (C3) levels decreased to below the lower limit of normal and then increased toward baseline values in long-term treatment (Supplementary Fig. 3).

The data suggest that antibody responses impacted the safety profile

of pegvaliase. HAEs occurred most frequently in early treatment (< 6 months) when the immune response comprised predominantly of PEG IgM, PEG IgG, and PAL IgM antibodies and mean C3 and C4 levels were decreasing. The frequency of HAEs decreased in late treatment (> 6 months) as mean PEG antibody titers decreased to baseline levels, PAL antibody titers remained stable, and C3/C4 levels increased. Although temporally associated, no particular antibody analyte or titer level was predictive of an HAE or acute systemic hypersensitivity event for an individual participant.

Despite the observed increase in antibody titers as described above, no events suggesting immune complex-mediated end-organ damage related to pegvaliase were observed.

4. Discussion

In the PRISM clinical program, pegvaliase self-administered by

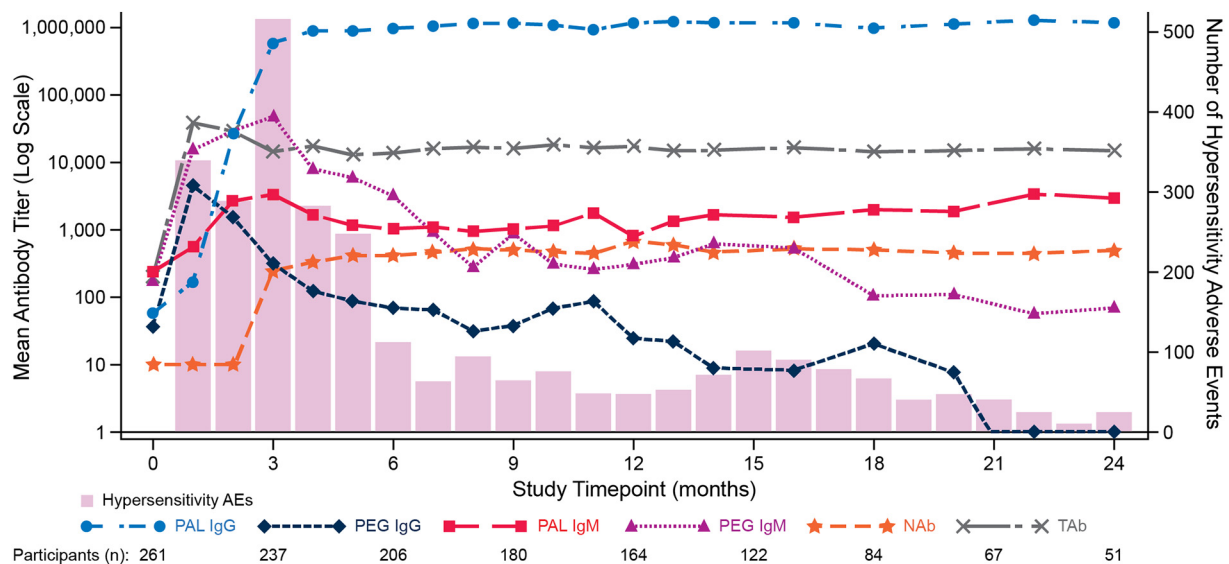


Fig. 7. Frequency of hypersensitivity adverse events and mean antibody titers over time (intent-to-treat population = 261). Sample size reflects participants with hypersensitivity adverse event data available at study timepoint and who have reached study timepoint at data cut; study is ongoing. IgG, immunoglobulin G; IgM, immunoglobulin M; PAL, phenylalanine ammonia lyase; PEG, polyethylene glycol; NAb, neutralizing antibodies; TAB, total antibody.

adults with PKU according to an induction, titration, and maintenance treatment regimen was associated with substantial reductions in blood Phe concentration, and was associated with a manageable safety profile. These durable reductions in blood Phe were temporally associated with improvements in symptoms of inattention and mood. Based on the descriptive summary of data from PRISM-1 and PRISM-2 trials described here, treatment with pegvaliase was associated with sustained and substantial blood Phe reductions over a 24-month follow-up period, with 61% of participants achieving the ACMG guideline-recommended blood Phe concentration of $\leq 360 \mu\text{mol/L}$ and 51% of participants reaching normal blood Phe levels of $\leq 120 \mu\text{mol/L}$ by 24 months of treatment [1]. The efficacy of pegvaliase to lower blood Phe to normal, unaffected population blood Phe levels ($60\text{--}120 \mu\text{mol/L}$) may provide a substantial benefit to adults with PKU, though more studies are needed. Sustained improvements from baseline were seen in inattention measured by ADHD RS-IV IA, with a greater magnitude of improvement observed in symptomatic participants with baseline ADHD RS-IV IA subscale scores > 9 . These findings are consistent with previous studies reporting a correlation between improved cognitive symptoms in adults with PKU and improvement in blood Phe control [32,33].

Although participants were required to maintain their natural protein intake during the studies, specific restrictions for natural protein consumption were not part of the inclusion/exclusion criteria for PRISM-1 and the resulting study population reported consuming amounts of protein much higher than the severe dietary Phe restriction recommended in PKU patients (estimated 6 g of natural protein intake is typically allowed in PKU patients with severe disease). This finding is consistent with several studies that report strict dietary restrictions to be ineffective or not feasible long-term in adults with PKU [4,6,7]. Study participants achieved a substantial reduction in blood Phe concentration, with total protein intake relatively stable throughout the study and mean increases from baseline in daily dietary Phe intake. Notably, the observed increases in dietary Phe could have blunted the blood Phe-reducing effect of pegvaliase, and blood Phe reductions may have been even greater in the absence of increased dietary Phe intake.

Elevated blood Phe in adults with PKU is associated with neuropsychological and neurocognitive deficits that present significant social, interpersonal, and work-related challenges that negatively impact daily function and quality of life [2–4,34]. The data presented here are consistent with previous studies reporting a correlation between improved neuropsychiatric symptoms in adults with PKU and improvement in blood Phe control, suggesting that neuropsychiatric deficits are at least partially reversible with reduction in blood Phe [3].

The majority of AEs were mild to moderate, with arthralgia and ISR reported most frequently. Approximately one-third of participants discontinued treatment during the first two years of pegvaliase treatment, and of these, half discontinued due to an AE. Overall, the AE profile appeared to improve with long-term treatment, with event rates decreasing from 58.6 per person-year in early treatment (< 6 months) to 19.4 per person-year in late treatment (> 6 months). Half of the participants who experienced acute systemic hypersensitivity events continued treatment after the event and all events resolved without sequelae. A reduction in severe events was seen following implementation of additional safety procedures to mitigate HAEs during the study. These safety procedures, in addition to the increased education of patients and physicians on AEs, likely contributed to the lower rates of AEs and discontinuations due to AEs as the PRISM study continued.

Because pegvaliase is a bacterial-derived protein, immunologic AEs, such as HAEs, were expected [35,36]. The most clinically important AEs were acute systemic hypersensitivity events consistent with NIAID/FAAN clinical anaphylaxis criteria, which had a lower event rate in late treatment (> 6 months) compared to early treatment (< 6 months). Acute systemic hypersensitivity events were not associated with IgE.

Participants treated with pegvaliase developed antibodies against the PAL enzyme as well as against the PEG moiety conjugated to PAL. The sustained antibody response against PAL followed a predictable T

cell-dependent antibody response, commonly mounted against foreign proteins [35], and the transient antibody response against PEG was typical of a T cell-independent type 2 antibody response [36]. HAEs occurred more often in early treatment (< 6 months) when the immune response was still immature and was composed predominantly of peaking levels of PEG IgM, PEG IgG, and PAL IgM antibodies along with declining complement levels. The frequency of HAEs decreased in late treatment (> 6 months) as the immune response matured, the PEG antibody responses were lost, PAL IgG antibodies became most prevalent, and C3/C4 levels increased toward baseline. The antibody response in early treatment (< 6 months) was comprised predominantly of pentameric IgM (both PEG IgM and PAL IgM), which is more efficient at complement activation than the antibody response in late treatment (> 6 months), composed predominately of IgG (only PAL IgG) [36–39]. Furthermore, the primarily PAL IgG antibodies of the immune response in late treatment are likely less able to form complement-fixing immune complexes than the PEG antibodies of early treatment due to PAL epitope masking by the extensive PEGylation on the surface of the drug product. The observed decreases in C4 levels are consistent with immune complex-mediated activation of the complement pathway. The totality of the antibody data and the lack of IgE detection suggest that the predominant mechanism of hypersensitivity reactions is type III immune complex-mediated hypersensitivity.

The open-label design and use of neuropsychiatric tools that rely on self-reporting may introduce biases into the study results reported here. Self-reporting tools are essential to capturing patients' experiences from their own perspective, particularly with regard to internal states such as mood and anxiety. However, patients' capacity to report on their own cognitive ability may be limited when self-awareness is affected by changes in blood Phe [40]. The use of the investigator-administered ADHD-RS IV provided a more objective assessment of attention, but is still based on patient interview. Performance-based neurocognitive measurement tools may provide better measurement of executive functioning. Another potential limitation in measuring the efficacy of pegvaliase in early treatment was the initiation of pegvaliase using randomized and fixed maintenance doses, rather than adjusted doses to achieve individualized and clinically meaningful blood Phe concentrations.

5. Conclusions

To date, pegvaliase has been studied in > 350 adult patients with PKU across 7 clinical trials, including the PRISM trials, the largest Phase 3 clinical programs conducted in adult patients with PKU. In the PRISM clinical program, pegvaliase self-administered with an induction, titration, and maintenance treatment regimen in adults with PKU was associated with sustained reductions in blood Phe concentration that were associated with sustained improvements in symptoms of inattention, with a manageable safety profile for most subjects. The results of the PRISM trials presented here and also by Harding et al. [23], who recently reported on the RDT portion of PRISM-2, underscore the potential of pegvaliase to profoundly change the treatment of PKU and improve long-term clinical outcomes.

Though PKU is one of the most well-studied inborn errors of metabolism, patients are often unable to achieve blood Phe control with current therapies and suffer significant morbidity. Pegvaliase is a promising novel investigational therapy that addresses the underlying metabolic cause of PKU by reducing blood Phe levels, with many participants in the pegvaliase clinical trials able to achieve blood Phe levels $< 360 \mu\text{mol/L}$ with increased intake of natural protein. The open-label extension of the Phase 3 study is ongoing and will continue to assess the long-term efficacy and safety of pegvaliase.

Acknowledgements

The authors would like to thank PRISM study investigators, study

coordinators and study site support staff; Dr. Stacey Tobin, representing MedAccess, for providing medical writing and editorial assistance; and Dr. Vanessa Birardi (BioMarin) and Kelly Lau (BioMarin) for critical review of the manuscript; Dr. Suyash Prasad and Dr. Saba Sile for overseeing portions of the study and contributing to the design. Funding for this support was provided by BioMarin Pharmaceutical Inc.

Contributions

MM was the medical monitor. ZG performed the statistical analysis. All authors interpreted the results, reviewed and contributed to the manuscript and were fully responsible for the content and editorial decisions related to this manuscript.

Disclosures

JT, HL, SA, JV, RZ, DD, COH and HN are investigators in clinical trials sponsored by BioMarin Pharmaceutical Inc. JT participated in advisory boards and received research support (BioMarin). HL is an advisory board member (BioMarin). JV received research support (BioMarin). RZ is a consultant (BioMarin, Hyperion). DD has received research support and consulting fees (BioMarin). CH has received research support and consulting fees (BioMarin). DAB has participated in advisory boards and received consulting fees (BioMarin). HN has participated in advisory boards and received consulting fees (BioMarin). HHW, JO, MM, JJ, KL, SG, and ZG are BioMarin Pharmaceutical Inc. employees and stockholders.

Funding

This work was supported by BioMarin Pharmaceutical Inc., Novato, CA.

Appendix A. Investigators and study sites

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Appendix B. Supplementary data

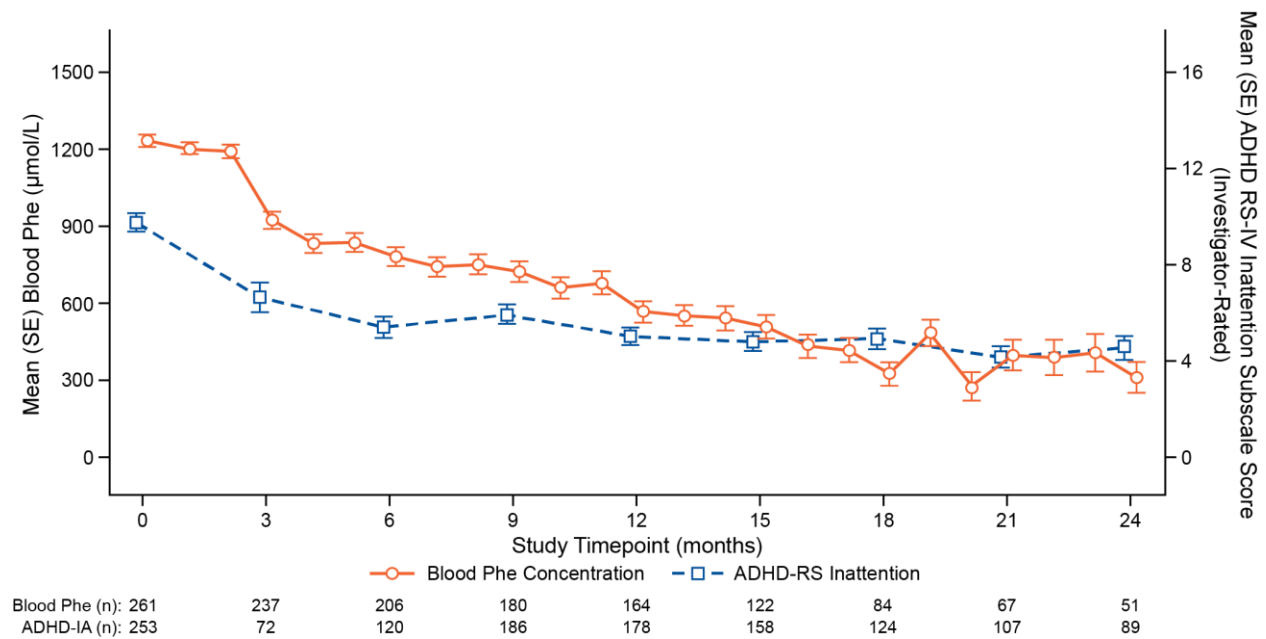
Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ymgme.2018.03.006>.

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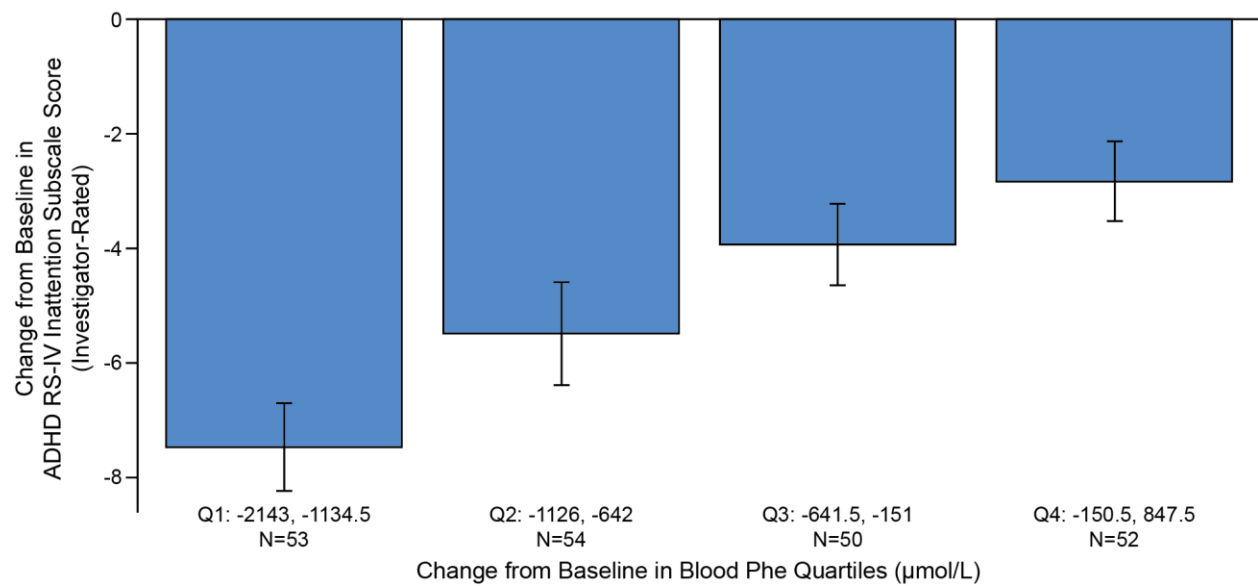
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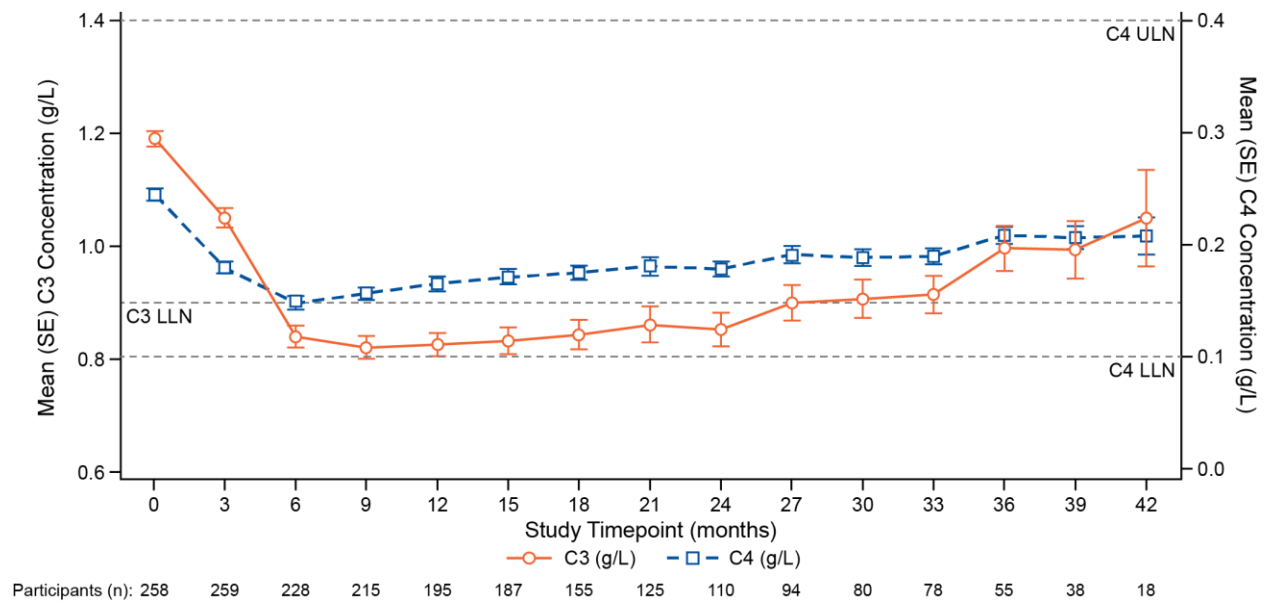
SUPPLEMENTARY MATERIALS



Supplementary Figure 1. Mean (SE) blood Phe concentration and mean (SE) investigator-rated ADHD RS-IV IA scores (intent-to-treat population, N=261). Sample size reflects participants with data available at study timepoint and who have reached study timepoint at data cut; study is ongoing. ADHD RS-IV IA, Attention Deficit Hyperactivity Disorder Rating Scale IV inattention subscale; SE, standard error.



Supplementary Figure 2. Change from baseline in ADHD RS-IV IA subscore by change from baseline in blood Phe quartiles at last observation (N=209). Sample size reflects participants with data available at baseline and at least one follow-up measure by data cut; study is ongoing. Blood Phe measurement used the average of the last 2 blood Phe measurements on or before the last ADHD RS-IV IA score collection date. ADHD RS-IV IA, Attention Deficit Hyperactivity Disorder Rating Scale IV inattention subscale.



Supplementary Figure 3. Complement C3 and C4 levels over time. Normal range for C3 is 0.9 to 1.8 g/L. Normal range for C4 is 0.1 to 0.4 g/L. C3, complement component 3; C4, complement component 4; LLN, lower limit of normal; SE, standard error; ULN, upper limit of normal.

Supplementary Table 1. Pegvaliase Dosing Regimen

	Study Period	Duration	Pegvaliase Dose	Frequency of Administration Each Week	Total Weekly Dose
PRISM-1	Induction	4 weeks	2.5 mg ^a	1	2.5 mg
	Titration	5 to 30 weeks	2.5 mg	2 ^b	5 mg
			10 mg	1	10 mg
			10 mg	2 ^b	20 mg
			10 mg	4	40 mg
			10 mg	7	70 mg
			20 mg	7	140 mg
	Maintenance	24 to 36 weeks ^c	20 or 40 mg	7	140 or 280 mg
PRISM-2	Part 1	13 weeks ^d	20 or 40 mg	7	140 or 280 mg
	Part 2	8 weeks	Placebo or 20 mg	7	0 or 140 mg
	Part 3	6 weeks	Placebo or 40 mg	7	0 or 280 mg
	Part 4	Up to 212 weeks ^e	20 or 40 mg	7	140 or 280 mg
			5 to 60 mg	7	35 to 300 mg

^aSelf-administration of at least first 2 doses performed in clinic. ^bRecommended to separate doses by at least 1 day. ^cParticipants remained on maintenance doses for at least 24 weeks.

^dParticipants who reached the maintenance dose in PRISM-1 transitioned to Part 1 of PRISM-2.

^eParticipants who did not reach the maintenance dose in PRISM-1 transitioned directly to Part 4 of PRISM-2.

Supplementary Table 2. Pegvaliase Discontinuation in Early (First 6 Months) Treatment Before and After Implementation of Additional Safety Procedures (intent-to-treat population, N=261)

	Enrolled Before Implementation (N=143)	Enrolled After Implementation (N=118)
Discontinued pegvaliase early, n (%)	34 (23.8%)	16 (13.6%)
Reason for pegvaliase discontinuation, n (%)		
Adverse event	22 (15.4%)	7 (5.9%)
Lost to follow-up	0	1 (0.8%)
Other	0	1 (0.8%)
Physician decision	3 (2.1%)	1 (0.8%)
Pregnancy	1 (0.7%)	0
Protocol deviation	1 (0.7%)	0
Withdrawal by participant	7 (4.9%)	6 (5.1%)

Supplementary Table 3. Daily Dietary Protein and Phe Intake (intent-to-treat population, N=261)

	Baseline (n=250)	6 Months (n=209)	12 Months (n=160)	18 Months (n=75)	24 Months (n=49)
Total dietary protein intake ^a , g/day					
Mean (SD)	64.8 (32.2)	68.5 (27.9)	71.6 (24.3)	71.2 (25.9)	77.4 (20.8)
Dietary protein intake from medical food, g/day					
Mean (SD)	26.3 (28.5)	26.0 (27.4)	24.2 (26.0)	15.6 (21.9)	18.4 (25.2)
Dietary Phe intake, mg/day					
Mean (SD)	1700.2 (1194.4)	1917.9 (1157.7)	2123.2 (1302.2)	2482.7 (1162.3)	2679.7 (1285.7)

SD, standard deviation. Baseline was defined as the last measurement before the first dose of pegvaliase (i.e., treatment-naïve) in the parent study. Protein intake was calculated as the daily average intake over 3 days prior to the assessment point. Phe intake was calculated as the daily average intake over 3 days prior to the assessment point. ^a Total daily dietary protein intake included protein from medical food and natural food.

Supplementary Table 4. Adverse Events by Preferred Term With $\geq 15\%$ Incidence Rate (intent-to-treat population, N=261; 401.3 person-years total treatment exposure)

	Incidence of AEs, n (%)	Event Rate per Person-Year
AE	261 (100.0%)	30.7
Arthralgia	184 (70.5%)	2.6
Injection-site reaction	162 (62.1%)	4.3
Injection-site erythema	125 (47.9%)	1.7
Headache	123 (47.1%)	1.6
Nasopharyngitis	91 (34.9%)	0.46
Rash	90 (34.5%)	0.74
Injection-site pruritus	86 (33.0%)	0.77
Injection-site bruising	84 (32.2%)	0.69
Injection-site pain	71 (27.2%)	0.55
Injection-site swelling	64 (24.5%)	0.61
Fatigue	61 (23.4%)	0.35
Injection-site rash	52 (19.9%)	0.44
Pain	49 (18.8%)	0.19
Pyrexia	45 (17.2%)	0.18

AE, adverse event. Event rate was calculated as total number of events divided by person-years of exposure.

Supplementary Table 5. Adverse Event Overview Before and After Implementation of Additional Safety Procedures by Treatment Phase, Reported as Event Rate (events/person-years) and Total Number of Events, (intent-to-treat population, N=261)

	Early Treatment Phase (≤6 Months)	Late Treatment Phase (>6 Months)				Total
		>6 to ≤12 Months	>12 to ≤24 Months	>24 to ≤36 Months	>36 Months	
Before implementation of additional safety procedures (N=143)						
Participants, N	N=143	N=108	N=93	N=78	N=23	N=108
Total treatment exposure, person-years ^a	61.3	48.3	83.1	58.6	3.5	193.6
Event rate, per person-year (total number of events)						
AEs	57.6 (3526)	25.3 (1221)	19.4 (1615)	13.4 (786)	17.6 (61)	19.0 (3683)
SAEs	0.3 (16)	0.3 (13)	0.1 (11)	0.03 (2)	0	0.1 (26)
HAEs	16.0 (982)	4.1 (199)	3.5 (294)	2.0 (119)	1.2 (4)	3.2 (616)
Acute systemic hypersensitivity events	0.1 (3)	0.02 (1)	0.01 (1)	0.02 (1)	0	0.02 (3)
Injection-site reactions	24.0 (1470)	5.7 (274)	3.9 (320)	2.1 (122)	4.9 (17)	3.8 (733)
Injection-site skin reactions lasting ≥14 days	1.5 (93)	0.8 (38)	0.8 (64)	0.3 (20)	0	0.6 (122)
Generalized skin reactions lasting ≥14 days	0.6 (39)	0.2 (11)	0.4 (30)	0.2 (10)	0.3 (1)	0.3 (52)
Arthralgia	7.0 (431)	1.1 (54)	0.8 (62)	0.3 (20)	0.3 (1)	0.71 (137)
After implementation of additional safety procedures (N=118)						
Participants, N	N=118	N=102	N=95	N=10	N=0	N=102
Total treatment exposure, person-years ^a	54.4	48.4	42.2	1.2	0	92.0
Event rate, per person-year (total number of events)						
AEs	59.7 (3248)	22.2 (1073)	18.5 (779)	13.2 (16)	0	20.3 (1868)
SAEs	0.2 (10)	0.1 (7)	0.1 (2)	0	0	0.1 (9)
HAEs	15.1 (823)	4.4 (212)	7.5 (316)	0.8 (1)	0	5.8 (529)
Acute systemic hypersensitivity events	0.1 (3)	0.2 (8)	0	0	0	0.1 (8)
Injection-site reactions	27.3 (1483)	6.6 (321)	2.6 (108)	1.7 (2)	0	4.7 (431)
Injection-site skin reactions lasting ≥14 days	1.1 (60)	0.6 (28)	0.3 (11)	0	0	0.42 (39)

Generalized skin reactions lasting ≥14 days	1.0 (54)	0.4 (21)	0.1 (6)	0	0	0.3 (27)
Arthralgia	6.7 (363)	1.9 (93)	0.7 (28)	0	0	1.3 (121)

AE, adverse event; HAE, hypersensitivity AE; SAE, serious AE. For each treatment phase and time interval, only AEs with onset within that phase or interval were included. Event rate was calculated as total number of events divided by person-years of exposure. ^a Total treatment exposure was the aggregated duration of treatment across all participants (for each participant, time from the first dose to 30 days after the last dose administered across all studies in which the participant was enrolled). Intervals of missing doses that were >28 consecutive days were excluded from the calculation of treatment duration.