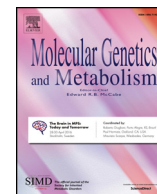




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Induction, titration, and maintenance dosing regimen in a phase 2 study of pegvaliase for control of blood phenylalanine in adults with phenylketonuria

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ABSTRACT

Background: Phenylketonuria (PKU) is caused by a deficiency in phenylalanine hydroxylase enzyme activity that leads to phenylalanine (Phe) accumulation in the blood and brain. Elevated blood Phe levels are associated with complications in adults, including neurological, psychiatric, and cognitive issues. Even with nutrition and pharmacological management, the majority of adults with PKU do not maintain blood Phe levels at or below guideline recommended levels. Pegvaliase, PEGylated recombinant *Anabaena variabilis* phenylalanine ammonia lyase (PAL), converts Phe to trans-cinnamic acid and ammonia, and is an investigational enzyme substitution therapy to lower blood Phe in adults with PKU.

Methods: Pegvaliase was administered using an induction, titration, and maintenance dosing regimen in adults with PKU naïve to pegvaliase treatment. Doses were gradually increased until blood Phe $\leq 600 \mu\text{mol/L}$ was achieved. The maintenance dose was the dose at which participants achieved and sustained blood Phe $\leq 600 \mu\text{mol/L}$ for at least 4 weeks without dose modification. Analyses were performed for participants who achieved (Group A, $n = 11$) and did not achieve (Group B, $n = 13$) maintenance dose during the first 24 weeks of study treatment.

Results: Baseline mean blood Phe for Group A and Group B were $1135 \mu\text{mol/L}$ and $1198 \mu\text{mol/L}$, respectively. Mean blood Phe $\leq 600 \mu\text{mol/L}$ was achieved for Group A by Week 11 (mean blood Phe of $508 \pm 483 \mu\text{mol/L}$) and for Group B by Week 48 (mean blood Phe of $557 \pm 389 \mu\text{mol/L}$). The most common adverse events involved hypersensitivity reactions, which were mostly mild to moderate in severity and decreased over time. One participant in Group B had four acute systemic hypersensitivity events of anaphylaxis consistent with clinical National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network criteria; all events were non-IgE mediated and resolved without sequelae, with pegvaliase dosing discontinued after the fourth event. The incidence and titers of anti-drug antibodies were generally lower in Group A compared to Group B.

Conclusions: Pegvaliase administered with an induction, titration, and maintenance dosing regimen demonstrated substantial efficacy at reducing blood Phe in both Group A and Group B by Week 48, with a manageable safety profile in most participants. Blood Phe reduction due to pegvaliase appears to be related to dose, treatment duration, and individual immune response; given additional time on treatment and dose titration, later Phe responders (Group B) achieved benefit similar to early Phe responders (Group A), with similar long-term safety profiles.

Abbreviations: μg , Microgram; μmol , Micromol; ACMG, American College of Medical Genetics and Genomics; ADA, Anti-drug antibody; AE, Adverse event; BH₄, Tetrahydrobiopterin; C3, Complement Component 3; C4, Complement Component 4; CTCAE, Common Terminology Criteria for Adverse Events; HAE, Hypersensitivity adverse event; IgE, Immunoglobulin E; IgG, Immunoglobulin G; IgM, Immunoglobulin M; kg, Kilogram; L, Liter; LLN, Lower limit of normal; MedDRA, Medical Dictionary for Regulatory Activities; mg, Milligram; N or n, Sample size; NAb, Neutralizing antibodies; NIAID/FAAN, National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network; PAH, phenylalanine hydroxylase; PAL, phenylalanine ammonia lyase; PEG, Polyethylene glycol; Phe, phenylalanine; PKU, phenylketonuria; SMQ, Standardized MedDRA Queries

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1. Introduction

Phenylketonuria (PKU) is an autosomal-recessive inborn error of phenylalanine (Phe) metabolism, in which deficiency or total loss of the phenylalanine hydroxylase (PAH) enzyme results in decreased conversion of Phe to tyrosine in the body leading to Phe accumulation in the blood and brain [1]. If untreated, PKU results in severe intellectual disability in children [2] and is associated with cognitive dysfunction, memory impairment, and behavior and psychiatric problems, such as depression and anxiety, in adults [3, 4]. In the United States, the incidence of PKU is estimated to be 1 in 16,500 newborns [5].

PKU is treated with severe restriction of dietary Phe (found in most natural protein foods) supplemented by Phe-free amino acid-modified medical foods and special low-protein foods [6]. There are numerous barriers to dietary compliance including inconvenience, poor social functioning, cost, time, access, availability, and the stigma of PKU [7, 8]. A growing body of evidence indicates that, even with severe restriction of Phe intake and supplementation of medical foods, neurocognitive and psychosocial outcomes are suboptimal in individuals with PKU [9–11]. In addition, the majority of adults have blood Phe levels above the recommended range [12] and many are not being followed in a metabolic clinic [8]. An estimated 50% of patients with PKU are lost to follow-up, which increases to 70% when only considering adults [8]. PAH management guidelines from the American College of Medical Genetics and Genomics (ACMG), published in January 2014, recommend life-long treatment of PKU, with a goal of maintaining blood Phe in the range of 120–360 $\mu\text{mol/L}$ [6]. Many experts treating PKU are also considering lower treatment targets to within the normal range of blood Phe 60–120 $\mu\text{mol/L}$.

Pharmacologic therapy with sapropterin dihydrochloride (sapropterin, KUVAN®, BioMarin Pharmaceutical Inc., Novato, CA) was approved in 2007 to lower blood Phe levels in responsive individuals with PKU in combination with Phe restriction. Sapropterin is an oral synthetic formulation of tetrahydrobiopterin (BH_4), an essential cofactor of PAH that is dependent on residual PAH activity. In clinical trials, only approximately 20% to 56% of patients with PKU responded to treatment with sapropterin [13–15], highlighting a significant unmet clinical need for treatment options to help achieve blood Phe control in a substantial proportion of individuals with PKU.

In 1999, phenylalanine ammonia lyase (PAL), a Phe-metabolizing enzyme found in plants, fungi, and bacteria, was suggested as a possible treatment for PKU [16]. PAL breaks down Phe into metabolically insignificant amounts of ammonia and the innocuous metabolite *trans*-cinnamic acid, which are then metabolized or excreted [17]. Although PAL functions without exogenous cofactors, it does require protection from degradation by proteases, as well as shielding from immune system recognition by pegylation [16]. Pegvaliase is recombinant *Anabaena variabilis* PAL that has been pegylated for development as a potential enzyme substitution therapy for PKU [18, 19].

In the first in-human study of pegvaliase, adults with PKU receiving doses ranging from 0.001 to 0.03 mg/kg did not achieve meaningful Phe reduction; however, a single dose of 0.1 mg/kg was tolerated and resulted in a mean 54% reduction in blood Phe concentration for up to 6 days post injection [20]. Similarly, in early phase 2 studies, once-weekly dosing at 0.001 to 0.1 mg/kg was tolerated, but did not result in significant blood Phe reductions; five-day-a-week dosing at 0.06 to 0.8 mg/kg/day resulted in transient blood Phe reductions, but was associated with a peak in hypersensitivity adverse events (HAEs) requiring dose interruptions or reductions [21]. An additional phase 2 study was performed to evaluate a pegvaliase dosing regimen that optimizes safety and achieves durable efficacy in a timely manner. We report the outcomes of this phase 2 study evaluating pegvaliase dosed in an induction, titration, and maintenance regimen in adults with PKU.

2. Methods

2.1. Study design

The phase 2 165-205 study (NCT01560286, conducted May 2012–Sept 2013) was a 24-week, multicenter, open-label, dose-finding study to evaluate the efficacy, safety, and immune response of subcutaneously administered pegvaliase in adults with PKU who were naïve to pegvaliase treatment. Participants who completed the 24-week study were eligible to continue pegvaliase therapy in a phase 2, open-label, long-term extension study, PAL-003 (NCT00924703, ongoing; data as of Oct 2016 reported). The extension study (starting from Week 25) evaluated the efficacy, safety, and immune response associated with long-term pegvaliase treatment [22].

Participants were instructed to maintain a stable diet with no modifications in total protein intake (from medical food or natural proteins) during the 4 weeks preceding the first dose of pegvaliase through study end. At the investigator's discretion based on factors such as blood Phe and metabolic needs, in the case of hypophenylalanemia (blood Phe $\leq 30 \mu\text{mol/L}$ for a minimum of 1 month), participants continuing to experience low blood Phe after two dose reductions could eliminate medical foods under dietitian guidance.

2.2. Participants

Eligible participants were aged 16 to 70 years and had a diagnosis of PKU with blood Phe concentration $\geq 600 \mu\text{mol/L}$ at screening and an average blood Phe concentration $\geq 600 \mu\text{mol/L}$ in the 6 months prior to screening. Participants with prior sapropterin treatment were eligible if sapropterin had been discontinued ≥ 4 months prior to screening. Participants were not eligible if they used or planned to use other injectable drugs containing polyethylene glycol (PEG) within 3 months prior to screening or during the study, or if they had consumed large neutral amino acid medical foods within 2 days prior to starting pegvaliase. Other exclusion criteria were pregnancy, breastfeeding, or intention to become pregnant (participant or partner).

2.3. Induction-titration-maintenance regimen

Low-dose induction therapy of 2.5 mg/week pegvaliase was started for 4–8 weeks, after which the dose could be increased over a minimum of 4 weeks, in dose and frequency until reaching blood Phe $\leq 600 \mu\text{mol/L}$. The “maintenance dose” was defined as the dose resulting in blood Phe $\leq 600 \mu\text{mol/L}$ that was sustained for at least 4 weeks without dose modification. Once maintenance dose was achieved, participants entered the maintenance phase, where maintenance dosing was continued with a maximum of 75 mg/day given 5 days/week (375 mg/week); maximum frequency was revised to 7 days/week in a PAL-003 protocol change (June 2012). At any time, doses could be adjusted for safety and to attain or maintain blood Phe $\leq 600 \mu\text{mol/L}$. If a participant experienced a severe HAE, dosing was interrupted and/or adjusted until the participant recovered and then restarted at the same or a lower dose upon consultation between the investigator and the sponsor's medical monitor. In October 2014, a PAL-003 protocol change specified participants experiencing treatment-related acute systemic hypersensitivity events including potential events of anaphylaxis of Grade ≥ 3 per the National Cancer Institute's Common Terminology Criteria for Adverse Events (NCI-CTCAE) [23] and suspected to meet Brown's clinical criteria for a severe event (i.e., hypoxia, hypotension, or neurologic compromise) [24] could be permanently discontinued from study drug.

2.4. Assessments

The primary efficacy analysis was the change in blood Phe concentration from baseline through Week 24. Blood Phe concentration was measured at screening and weekly thereafter from Week 1 to Week 24 and then every 4 weeks thereafter. Fasting was required for at least 4 h prior to plasma collection. Blood Phe concentration was analyzed by a central laboratory (Quest Diagnostics Inc., San Juan Capistrano, CA), though a local laboratory could be used when necessary.

Safety analyses were conducted with evaluation of AEs, clinical laboratory assessments, vital signs assessments, and physical examinations. Vital signs, clinical laboratory (including complement panel), and adverse event assessments were evaluated at screening, weekly thereafter from Week 1 to Week 24 and then every 4 weeks thereafter. Abnormal results from laboratory or vital signs assessments were reviewed by the investigator for clinical significance and need for further intervention. All clinically significant results were reported as adverse events. Quantitative amino acids for Phe and tyrosine were assessed. AEs were evaluated at every clinic visit and coded by preferred term in accordance with the Medical Dictionary for Regulatory Activities (MedDRA) version 16.1 [25]. In addition, HAEs were identified using the modified version of the hypersensitivity standardized MedDRA query (SMQ) that included additional AE preferred terms of arthralgia, arthritis, eye inflammation, eye irritation, eye pain, joint stiffness, joint swelling, pyrexia, vision blurred, polyarthritis, and the broad algorithmic anaphylactic reaction SMQ. Potential acute systemic hypersensitivity events were reviewed by an allergist/immunologist independent of the clinical site and Sponsor to identify anaphylaxis events consistent with clinical criteria defined by the National Institute of Allergy and Infectious Diseases/Food Allergy and Anaphylaxis Network (NIAID/FAAN) and Brown's severe criteria [24, 26]. Injection-site reactions were identified using high-level term of injection-site reaction. Severity of each AE was determined by the investigator using NCI-CTCAE: Grade 1, mild; Grade 2, moderate; Grade 3, severe; Grade 4, life-threatening; Grade 5, death resulting from an AE [23].

Pegvaliase immunogenicity (secondary analysis) was measured using a panel of isotype-specific enzyme linked immunosorbent assays (ELISA) to detect antibodies directed against each component of the drug (PAL IgM, PAL IgG, PEG IgM, and PEG IgG). Radioallergosorbent test assays were used to detect IgE antibodies specific to pegvaliase or PAL. Testing for PAL IgM, PEG IgM, neutralizing antibodies (NAb), and IgE was performed using qualitative assays that yielded a positive or negative result; positivity was determined by assay signals greater than the assay cut point for each method, defined by the upper 95th percentile of the range of signals generated by a panel of sera from drug-naïve healthy adult volunteers. Testing for PAL IgG and PEG IgG was also performed using semi-quantitative assays that reported a titer for each analyte; patient serum samples with signals above the assay cut point were serially diluted until the signal fell below the assay cut point. The dilution factor of the last point with signal above the cut point was reported as the sample titer. Individual patient serum samples were collected for immunogenicity testing pre-dose at baseline, weekly from

Week 1 to Week 4, biweekly through Week 24, then monthly to quarterly thereafter, and within 48 h of any systemic hypersensitivity event. Immunogenicity analyses were performed by BioMarin, with the exception of IgE antibodies, which were analyzed by a contract research organization (Viracor-IBT Laboratories Inc., Lee's Summit, MO).

Dietary Phe and protein intakes (exploratory analyses) were assessed every 4 weeks using data from diet diaries, where participants recorded their dietary intakes for 3 days immediately preceding a clinic visit. Dietary intakes were analyzed with MetabolicPro software (Genetic Metabolic Dietitians International, Decatur, GA, USA). Daily dietary protein intake was calculated using three-day averages of dietary protein and Phe intake data.

2.5. Statistical analysis

Efficacy analyses included participants who received any amount of pegvaliase and had any post-treatment blood Phe concentration measurements. Analyses were performed for participants who achieved maintenance dose within 24 weeks (Group A) and participants who did not achieve maintenance dose within 24 weeks (Group B). The following parameters were summarized using descriptive statistics (mean, standard deviation [SD], minimum, and maximum): blood Phe concentration; change from baseline and percent change from baseline in blood Phe concentration; and achievement of blood Phe $\leq 120 \mu\text{mol/L}$, $\leq 360 \mu\text{mol/L}$, and $\leq 600 \mu\text{mol/L}$. To explore change in blood Phe concentration with respect to changes in diet, plots were generated for mean weekly blood Phe concentration and dietary Phe intake.

Safety analyses included all participants who received any amount of pegvaliase. AEs were summarized by preferred term and severity grade. For AEs that occurred more than once during the study, the AE with the maximum severity was reported in summaries. Exposure-adjusted AE rates were calculated to account for the longer time on treatment in the extension study (up to 204 weeks) than in 165–205 study (24 weeks).

3. Results

3.1. Participant Disposition and Characteristics

A total of 24 participants enrolled at 12 investigational sites in the United States and all participants completed the 24-week 165–205 study, with 2 participants discontinuing pegvaliase treatment early due to AEs. Group A (i.e., maintenance dose achieved in first 24 weeks of treatment) had 11 participants and Group B (did not achieve maintenance dose in first 24 weeks of treatment) had 13 participants. Twenty participants enrolled in the extension study, 10 from Group A and 10 from Group B, with 1 participant in each group discontinuing pegvaliase early in the extension study due to AEs (Fig. 1).

Mean age at enrollment (29 years) was similar for participants in Group A and Group B (Table 1). Mean baseline blood Phe levels were also comparable between Group A ($1135 \pm 348 \mu\text{mol/L}$) and Group B ($1198 \pm 243 \mu\text{mol/L}$). The overall demographic profile for participants

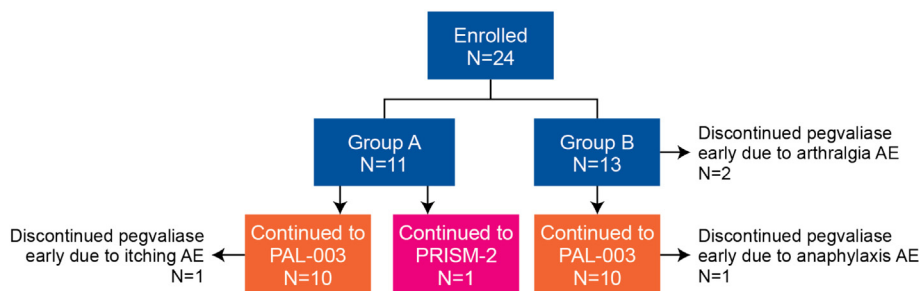


Fig. 1. Participant Disposition.

Table 1
Participant demographic and baseline characteristics.

	Early treatment (≤ 24 weeks)		Long-term treatment (> 24 weeks)
	Group A (n = 11)	Group B (n = 13)	All Participants (n = 20)
Age at enrollment			
Mean (SD), years	28.6 (12.3)	29.8 (11.1)	29.1 (10.8)
Participants ≥ 18 years, n (%)	10 (90.9%)	13 (100.0%)	19 (95.0%)
Participants < 18 years, n (%)	1 (9.1%)	0	1 (5.0%)
Female, n (%)	8 (72.7%)	5 (38.5%)	12 (60.0%)
Weight	n = 10	n = 13	n = 19
Mean (SD), kg	64.6 (17.7)	86.4 (25.3)	76.0 (24.7)
Body mass index	n = 10	n = 11	n = 17
Mean (SD), kg/m ²	23.6 (4.1)	29.4 (7.1)	26.5 (6.7)
Blood Phe concentration	n = 11	n = 13	n = 20
Mean (SD), $\mu\text{mol/L}$	1134.8 (348.3)	1197.5 (243.4)	1178.7 (296.3)
Median (min, max), $\mu\text{mol/L}$	1014.0 (713.0, 2021.0)	1238.0 (851.0, 1531.0)	1100.0 (713.0, 2021.0)
25th, 75th percentile, $\mu\text{mol/L}$	966.0, 1185.0	981.0, 1447.0	984.0, 1362.5
Total protein intake	n = 9	n = 11	n = 16
Mean (SD), g/day	59.7 (23.3)	71.3 (34.4)	70.3 (30.0)
Median (min, max), g/day	62.0 (11.7, 93.7)	70.3 (15.0, 138.3)	67.7 (11.7, 138.3)
Dietary Phe intake	n = 9	n = 11	n = 16
Mean (SD), mg/day	1354.9 (954.2)	1884.4 (1113.3)	1614.0 (1049.4)
Median (min, max), mg/day	959.7 (461.0, 3163.7)	1434.3 (713.7, 3515.7)	1047.7 (461.0, 3515.7)

Group A: achieved maintenance dose in first 24 weeks of treatment; Group B: did not achieve maintenance dose in first 24 weeks of treatment. Baseline was defined as the last measurement before the first dose of pegvalias (i.e., treatment-naïve) in the parent study. 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. Phe intake was 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 in a group. Max, maximum; Min, minimum; SD, standard deviation.

who continued into the extension study was similar to that of the 165–205 study.

A total of 24 participants enrolled and completed the parent study, 165–205, including 2 participants who discontinued pegvalias early but remained in the study for assessments. Twenty-one participants subsequently enrolled in the extension study, PAL-003, or a phase 3 study, PRISM-2, to continue pegvalias treatment. Group A included participants who reached maintenance dose (sustained blood Phe $\leq 600 \mu\text{mol/L}$ for at least 4 weeks) within 24 weeks of treatment. Group B included participants who did not reach maintenance dose within 24 weeks of treatment. AE, adverse event.

3.2. Pegvalias dosing

During the first 24 weeks of the study, the mean total weekly pegvalias dose was 64.7 mg and 89.4 mg for Group A and B, respectively, administered over 1 to 5 days per week. After Week 25 to data cut, the mean weekly doses ranged from 57.0 to 167.7 mg for Group A and 227.3 to 375.2 mg for Group B, administered with a frequency of up to 7 days per week. Pegvalias dose was consistently lower in Group A than in Group B (Fig. 2), including at Week 24 ($103 \pm 88 \text{ mg/week}$ vs. $231 \pm 136 \text{ mg/week}$) and Week 192 ($82 \pm 49 \text{ mg/week}$ vs. $227 \pm 152 \text{ mg/week}$) of treatment.

3.3. Blood Phe

With pegvalias treatment, 11 of 24 participants (46%) achieved maintenance dose during the first 24 weeks of the study (Group A) with mean blood Phe $\leq 600 \mu\text{mol/L}$ first observed at Week 11 (mean $508 \pm 483 \mu\text{mol/L}$), reflecting a mean reduction of $627 \pm 432 \mu\text{mol/L}$ (56% $\pm$ 36% reduction) from baseline. Mean blood Phe $\leq 600 \mu\text{mol/L}$ was maintained with long-term treatment (Fig. 2). The variability seen at Week 136 appears to be an artifact of decreased sample size. Among the 13 participants who did not achieve maintenance dose during the first 24 weeks of the study (Group B), 10 continued into the extension study; Group B achieved mean blood Phe $\leq 600 \mu\text{mol/L}$ by Week 48 of treatment, which was sustained during long-term treatment. At Week 48, mean blood Phe in Groups A and B was $236 \pm 308 \mu\text{mol/L}$ and $557 \pm 389 \mu\text{mol/L}$, respectively.

All participants in Group A achieved blood Phe $\leq 120 \mu\text{mol/L}$ during the first 24 weeks of the study (Table 2). In Group B, 3 out of 13 participants achieved blood Phe $\leq 600 \mu\text{mol/L}$ during the first 24 weeks of treatment and ultimately 9 out of 10 participants continuing with long-term treatment achieved blood Phe $\leq 600 \mu\text{mol/L}$, of whom 8 achieved blood Phe $\leq 120 \mu\text{mol/L}$. The one Group B participant in the extension that did not achieve Phe $\leq 600 \mu\text{mol/L}$ had discontinued pegvalias treatment early due to recurrent acute systemic hypersensitivity events consistent with clinical NIAID/FAAN anaphylaxis criteria (described in Section 3.5).

3.4. Dietary Phe and protein

For Group A, from baseline to Week 24, there were increases in mean dietary Phe ($1355 \pm 954 \text{ mg/week}$ to $1814 \pm 1316 \text{ mg/week}$; Fig. 3A) and dietary protein intakes (59.7 g/day to 68.2 g/day), which continued to increase in long-term treatment. For Group B, from baseline to Week 24, mean dietary Phe ($1884 \pm 1113 \text{ mg/week}$ to $1864 \pm 914 \text{ mg/week}$; Fig. 3B) and dietary protein (71.3 g/day to 67.0 g/day) intakes were relatively stable; however, increases occurred after Week 24 and continued in long-term treatment.

3.5. Adverse events

Overall, the exposure-adjusted AE rates were higher in the first 24 weeks of treatment. AE rates decreased in long-term treatment (after Week 24) for both Groups A and B (Table 3). Though AE rates were higher in Group B than Group A, the difference between groups decreased during long-term treatment, with rates for AEs and HAEs becoming comparable during the extension study. In the first 24 weeks of treatment, most participants had a Grade 2 AE (n = 19, 79%) as their highest severity AE (Grade 1, n = 4, 17%; Grade ≥ 3 AEs not reported). In the extension, most participants experienced AEs with highest severity of Grade 2 (n = 14, 70%) or Grade 1 (n = 5, 25%); the highest severity reported was Grade 3 (n = 1, 5%).

The AE profile for participants in the extension study was generally consistent with the first 24-week experience, with similar types of events occurring but with decreased incidence. The single exception was a higher rate of urticaria in the extension study, primarily

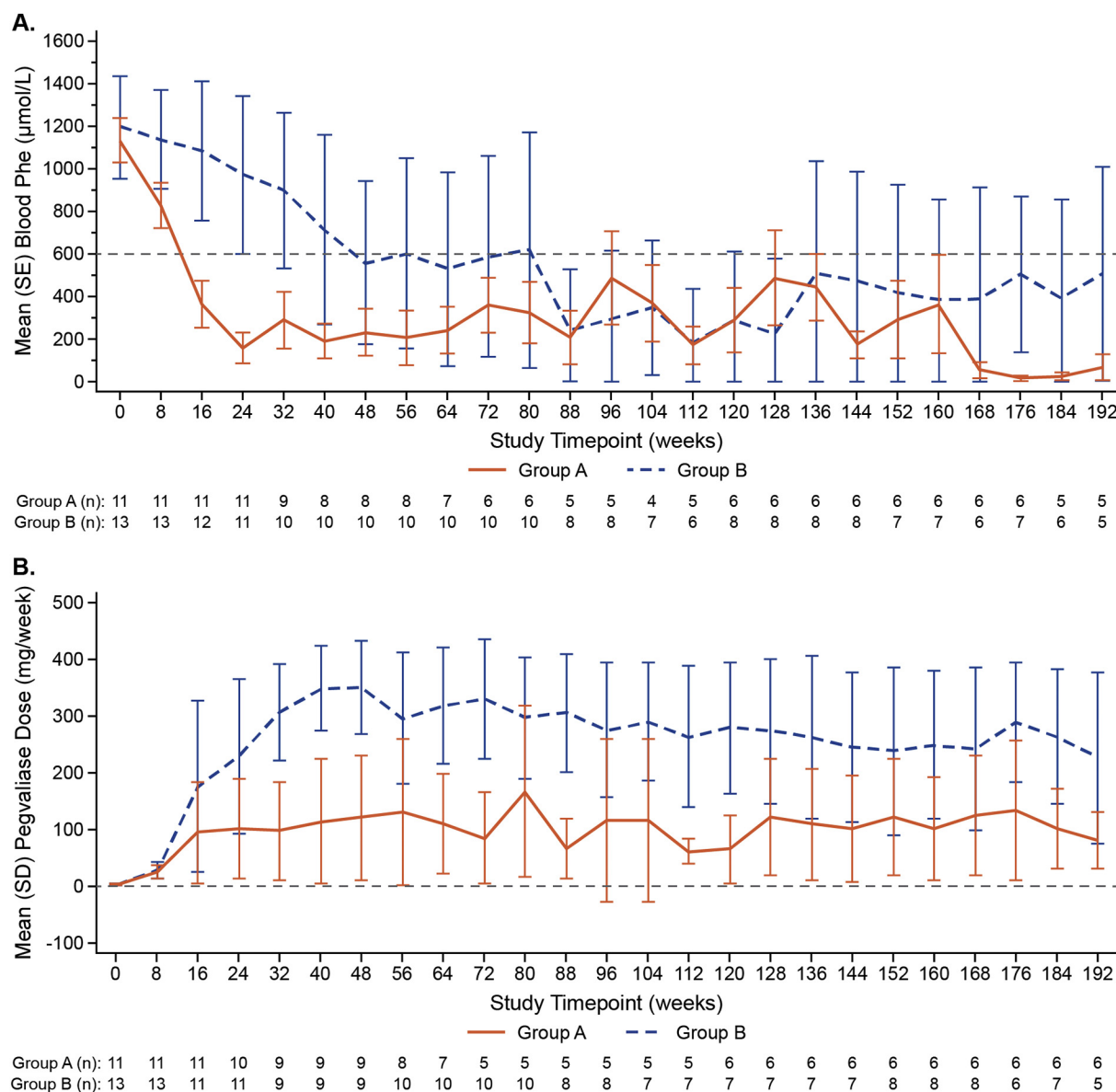


Fig. 2. Blood phenylalanine concentration (A) and pegvaliase dose (B) in Group A and Group B. Group A: achieved maintenance dose in first 24 weeks of treatment; Group B: did not achieve maintenance dose in first 24 weeks of treatment. Sample size reflects participants with data available at study timepoint at data cut; study is ongoing. SD, standard deviation; SE, standard error.

Table 2

Achievement of blood phenylalanine targets at any time point by participants in Group A and Group B.

Blood Phe Concentration, n (%)	Early Treatment (≤ 24 weeks)		Long-term Treatment (> 24 weeks)	
	Group A (n = 11)	Group B (n = 13)	Group A (n = 10)	Group B (n = 10)
$\leq 120 \mu\text{mol/L}$	11 (100%)	0	9 (90%)	8 (80%)
$\leq 360 \mu\text{mol/L}$	11 (100%)	0	10 (100%)	8 (80%)
$\leq 600 \mu\text{mol/L}$	11 (100%)	3 (23%)	10 (100%)	9 (90%)

Group A: achieved maintenance dose in first 24 weeks of treatment; Group B: did not achieve maintenance dose in first 24 weeks of treatment.

attributed to a single participant who reported 119 urticaria events (93% lasted one day; the longest lasted six days; and the participant continued pegvaliase treatment).

The most commonly reported AEs were arthralgia and injection-site

reactions during the first 24 weeks, and urticaria during the extension study (Table 4). The AE rate for injection-site reactions was approximately three times higher in Group B during the first 24 weeks and two times higher in Group B during the extension study, with $> 99\%$ of events requiring no dose change and $> 97\%$ of events resolving without sequelae.

Two Group B participants discontinued pegvaliase treatment within the first 24 weeks due to arthralgia AEs (joint ankle pain, Grade 1; worsening arthralgia, Grade 2) attributed to pegvaliase, but remained in the study for assessments. During long-term treatment, one participant in Group A discontinued pegvaliase due to an AE (itching, Grade 1 that persisted after drug discontinuation) but remained in the study for assessments.

One participant in Group B discontinued study treatment after a fourth acute systemic hypersensitivity event of anaphylaxis consistent with clinical NIAID/FAAN criteria; two of the events occurred in the first 24 weeks of treatment. The third and fourth events both occurred in long-term treatment and met Brown's severe criteria; all 4 events resolved without sequelae and were not associated with IgE positivity.

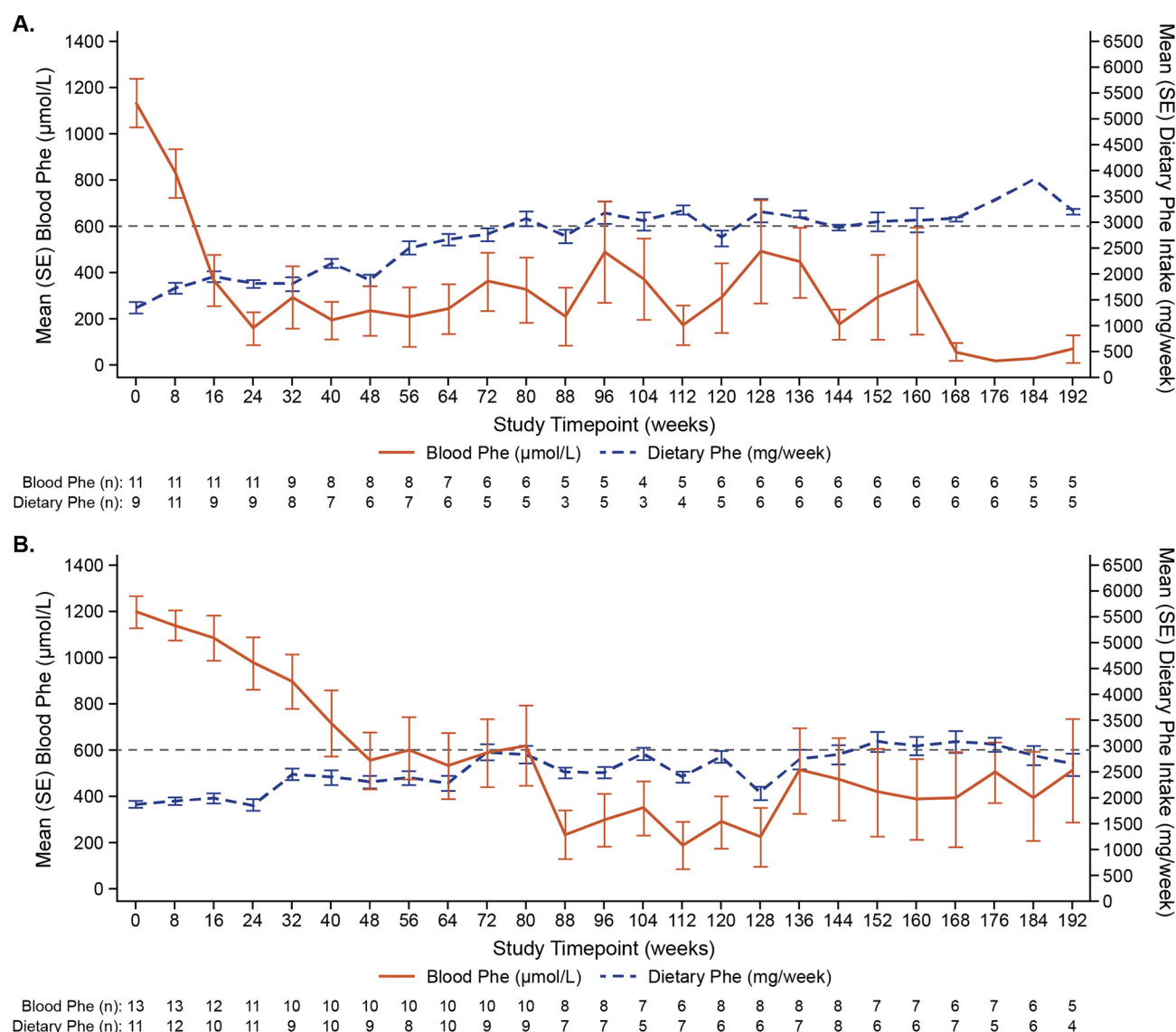


Fig. 3. Blood phenylalanine concentration and dietary phenylalanine intake in (A) Group A and (B) Group B. Group A: achieved maintenance dose in first 24 weeks of treatment; Group B: did not achieve maintenance dose in first 24 weeks of treatment. Sample size reflects participants with data available at study timepoint at data cut; study is ongoing. SD, standard deviation; SE, standard error.

The one serious AE (increase in blood creatinine phosphokinase associated with strenuous exercise two days prior, Grade 1) reported during the first 24 weeks was not considered drug-related by the investigator, resolved after dose interruption, and the participant continued with titration. One participant had two serious AEs in the extension study including a severe acute systemic hypersensitivity event of anaphylaxis (one of the events described above) and a Grade 2 HAE considered related to treatment. An additional serious Grade 2 HAE occurred during the extension study in another participant. All serious AEs resolved.

There was an initial decrease in mean complement component 3 (C3) and complement component 4 (C4) levels early treatment, which then trended upwards towards baseline with long-term treatment. Mean C3 levels decreased to below the lower limits of normal (LLN) in early treatment, then after reaching a low at 36 weeks, levels gradually increased into normal range by 96 weeks. Although there was an initial decrease from baseline, mean C4 levels remained above the LLN during the study. The initial decline in C3 and C4 levels occurred when PEG IgM, PEG IgG and PAL IgM incidence was at its highest, and then returned towards normal as the incidence of these antibodies decreased

(Supplementary Fig. 1). No other overall trends in laboratory, vital signs, or weight assessments that were clinically significant were reported.

3.6. Antibody responses

All participants developed antibody responses against the PAL protein, and a majority developed a transient antibody response against PEG during this study. Generally, incidence rates and mean titers of antibodies were lower in Group A compared to Group B. Incidence rates of PAL IgM were higher in Group B, and declined over time for both Groups A and B (Fig. 4A), whereas incidence and titer levels of PAL IgG responses increased in the first 24 weeks of treatment and then were stable in long-term treatment (Figs. 4C and 5). Anti-PEG responses declined to pre-treatment baseline levels over time in all participants (Fig. 4B, D). In early treatment, NAb were only detected in Group B participants (Fig. 4E). However, the majority of participants developed a NAb response during the extension study. No participants from this study confirmed positive for drug-specific IgE (PAL IgE or pegvaliase IgE) at or near the time of an acute systemic hypersensitivity event of

Table 3

Adverse event overview reported as incidence (n), event rate (events/person-years) and total number of events.

	Early Treatment (≤ 24 weeks)		Long-term Treatment (> 24 weeks)	
	Group A (n = 11)	Group B (n = 13)	Group A (n = 10)	Group B (n = 10)
Exposure, person-years	5.16	5.42	22.96	27.16
Incidence, event rate (total number of events)				
Adverse events	n = 10 35.63 (184)	n = 13 68.78 (373)	n = 10 15.63 (359)	n = 10 19.51 (530)
Related to study drug	n = 10 24.40 (126)	n = 13 59.38 (322)	n = 10 9.15 (210)	n = 10 13.92 (378)
Caused study drug discontinuation	0	n = 2 0.37 (2)	n = 1 0.04 (1)	n = 1 0.04 (1)
Caused study discontinuation	0	0	0	n = 1 0.04 (1)
Serious adverse events	0	n = 1 0.18 (1)	n = 1 0.04 (1)	n = 2 0.11 (3)
Related to study drug	0	0	0	n = 2 0.07 (2)
Caused study drug discontinuation	0	0	0	n = 1 0.04 (1)
Caused study discontinuation	0	0	0	n = 1 0.04 (1)
Hypersensitivity adverse events	n = 9 12.97 (67)	n = 13 18.44 (100)	n = 8 7.23 (166)	n = 9 7.44 (202)
Acute systemic hypersensitivity events of anaphylaxis	0	n = 1 0.37 (2)	0	n = 1 0.07 (2)
Injection-site reactions	n = 9 10.26 (53)	n = 13 30.43 (165)	n = 8 1.83 (42)	n = 8 3.79 (103)
Deaths	0	0	0	0

Group A: achieved maintenance dose in first 24 weeks of treatment; Group B: did not achieve maintenance dose in first 24 weeks of treatment. Event rate was calculated as total number of events divided by person-years of exposure.

anaphylaxis. The frequency of HAEs was highest in early treatment (Fig. 5) when incidence of PAL IgM and PEG IgM and incidence and titers of PEG IgG were highest. Frequency of HAEs decreased over time as the incidence of PEG IgM, PEG IgG and PAL IgM decreased, and PAL IgG reached steady state.

Group A: achieved maintenance dose in first 24 weeks of treatment; Group B: did not achieve maintenance dose in first 24 weeks of treatment. Sample size reflects participants with data available at study timepoint at data cut; study is ongoing. IgG, immunoglobulin G; IgM, immunoglobulin M; NAb, neutralizing antibodies; PAL, phenylalanine ammonia lyase; PEG, polyethylene glycol; SE, standard error.

Sample size reflects participants with hypersensitivity adverse event data available at study timepoint at data cut; study is ongoing. IgG, immunoglobulin G; IgM, immunoglobulin M; PAL, phenylalanine ammonia lyase; PEG, polyethylene glycol; NAb, neutralizing antibodies.

4. Discussion

Weekly, low-dose introduction of pegvaliase followed by gradual increases in dose and frequency led to clinically meaningful blood Phe reduction among adults with PKU who had elevated blood Phe (> 600 μmol/L) at baseline. Participants were started on low doses of pegvaliase that were gradually increased with the goal of achieving and maintaining an effective dose for each participant, while minimizing hypersensitivity events associated with initiating treatment, which were observed in earlier phase 2 studies [21, 22]. The majority of participants continuing into the extension study achieved and/or maintained blood Phe ≤ 600 μmol/L with pegvaliase treatment, though

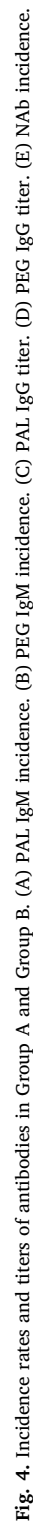
Table 4

Adverse events related to study drug with incidence ≥ 12.5% in any group reported by incidence (n), event rate (events/person-years), and total number of events.

	Early Treatment (≤ 24 weeks)		Long-term Treatment (> 24 weeks)	
	Group A (n = 11)	Group B (n = 13)	Group A (n = 10)	Group B (n = 10)
Exposure, person-years	5.16	5.42	22.96	27.16
Incidence, event rate (total number of events)				
Adverse event	n = 10 24.40 (126)	n = 13 59.38 (322)	n = 10 9.15 (210)	n = 10 13.92 (378)
Arthralgia	n = 7 5.23 (27)	n = 11 9.59 (52)	n = 4 0.91 (21)	n = 5 0.81 (22)
Injection site reaction	n = 5 3.49 (18)	n = 13 19.36 (105)	n = 3 0.13 (3)	n = 5 0.26 (7)
Injection site erythema	n = 2 1.94 (10)	n = 11 5.53 (30)	n = 4 0.39 (9)	n = 2 0.15 (4)
Rash	n = 3 1.16 (6)	n = 5 0.92 (5)	n = 5 1.00 (23)	n = 3 0.37 (10)
Urticaria	0	n = 3 1.11 (6)	n = 5 3.05 (70)	n = 5 4.79 (130)
Pruritus	n = 1 0.39 (2)	n = 4 0.74 (4)	n = 3 0.30 (7)	n = 2 0.15 (4)
Injection site pruritus	n = 3 1.36 (7)	n = 2 0.74 (4)	n = 4 0.44 (10)	0
Nausea	n = 3 0.77 (4)	n = 4 0.74 (4)	0	n = 2 0.11 (3)
Injection site bruising	n = 1 0.19 (1)	n = 4 0.74 (4)	n = 2 0.13 (3)	n = 1 0.04 (1)
Injection site rash	n = 4 2.13 (11)	n = 4 0.92 (5)	0	0
Peripheral swelling	0	n = 2 0.74 (4)	n = 3 0.22 (5)	n = 2 0.07 (2)
Headache	n = 1 0.19 (1)	n = 1 0.37 (2)	0	n = 4 0.44 (12)
Injection site pain	0	0	n = 2 0.13 (3)	n = 4 1.25 (34)
Rash generalized	n = 2 1.16 (6)	n = 3 0.74 (4)	0	n = 1 0.11 (3)
Alopecia	0	0	n = 2 0.09 (2)	n = 3 0.15 (4)
Contusion	0	n = 1 0.18 (1)	0	n = 4 0.33 (9)
Flushing	0	n = 4 0.92 (5)	0	n = 1 0.04 (1)
Injection site swelling	0	n = 3 0.92 (5)	n = 1 0.04 (1)	n = 1 0.11 (3)
Injection site urticarial	n = 1 0.19 (1)	n = 1 0.37 (2)	n = 1 0.35 (8)	n = 2 0.07 (2)
Anxiety	n = 2 0.39 (2)	n = 1 0.18 (1)	0	n = 1 0.15 (4)
Increased c-reactive protein	n = 1 0.19 (1)	n = 2 0.37 (2)	n = 1 0.04 (1)	0
Dyspnea	n = 1 0.19 (1)	n = 2 1.29 (7)	0	n = 1 0.07 (2)
Myalgia	0	n = 1 0.37 (2)	0	n = 3 0.15 (4)
Oropharyngeal pain	n = 1 0.19 (1)	0	0	n = 3 0.15 (4)
Pyrexia	0	n = 3 0.92 (5)	0	0
Vomiting	n = 1 0.19 (1)	n = 2 0.37 (2)	0	0

Group A: achieved maintenance dose in first 24 weeks of treatment; Group B: did not achieve maintenance dose in first 24 weeks of treatment. Event rate was calculated as total number of events divided by person-years of exposure.

there was variation in the dose and time on treatment required to achieve this goal due to variability in the immune response among participants. Participants with early treatment response (i.e., Group A) achieved mean Phe ≤ 600 μmol/L during the initial 24 weeks while those with later treatment response (i.e., Group B) achieved mean



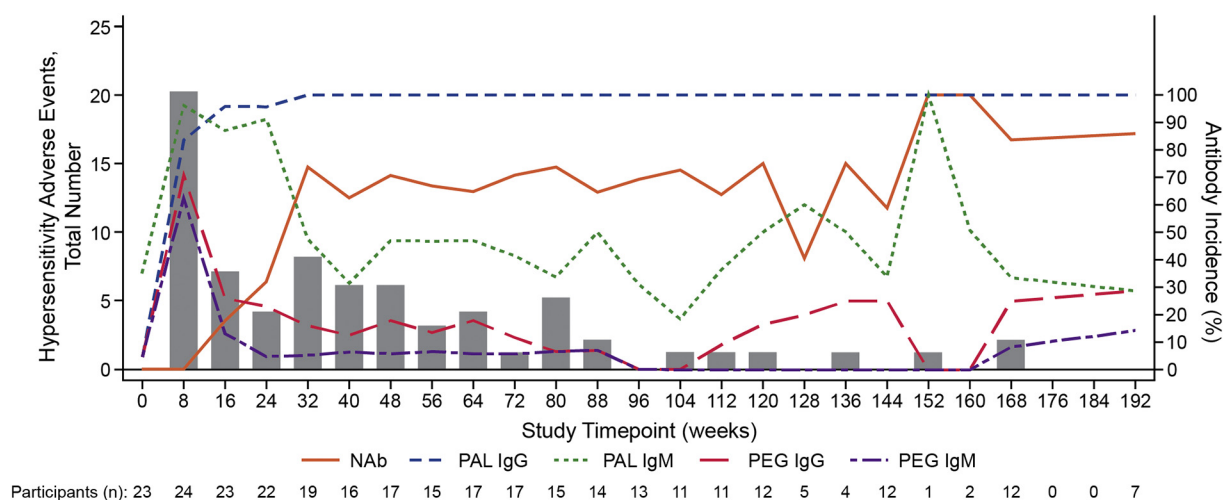


Fig. 5. Hypersensitivity adverse events and antibody incidence rate in all participants.

Phe $\leq 600 \mu\text{mol/L}$ by week 48; Group B required approximately 2–3 times the dose that Group A required to achieve guideline-recommended blood Phe concentrations. Mean dietary Phe intake was stable or increased in both groups concurrent with mean decreases in blood Phe, indicating that blood Phe reductions were not due to dietary manipulation.

The blood Phe target of $\leq 600 \mu\text{mol/L}$ was based on the National Institutes of Health PKU consensus statement on adult blood Phe recommendations in effect at the start of this study [27]. Despite targeting blood Phe of $\leq 600 \mu\text{mol/L}$ in this study, all Group A participants and 8 of 10 Group B participants who entered the extension study achieved blood Phe $\leq 360 \mu\text{mol/L}$, and 9 of 10 Group A participants and 8 of 10 Group B participants achieved blood Phe $\leq 120 \mu\text{mol/L}$. The potential efficacy of pegvaliase to provide a therapy that lowers blood Phe to levels found in the normal, unaffected population ($60\text{--}120 \mu\text{mol/L}$) may also provide significant benefit, though more studies are needed.

Pegvaliase administered to adults with PKU up to 7 times per week in an induction, titration, and maintenance dosing regimen had a manageable safety profile for most participants. Overall, AEs decreased with time on treatment and $> 99\%$ of events were mild to moderate, including HAEs, which were the most common AEs. Although there was a higher exposure-adjusted AE rate in Group B than Group A, the difference between groups decreased during long-term treatment with extended exposure to pegvaliase, maturation of the immune response, and in Group B, use of higher doses.

Given that PAL is a foreign, bacterially-derived protein, anti-drug antibody (ADA) responses to pegvaliase are expected [28, 29], and all participants treated with pegvaliase developed ADA against pegvaliase in the first 24 weeks. All participants developed antibodies against the PAL protein and a majority of participants developed transient antibodies against PEG. Variability in the magnitude of the immune response, reflective of intrinsic immune response differences among individuals, impacted the efficacy and safety of pegvaliase. The magnitude of the ADA response influenced the level of blood Phe reduction, likely through its impact on immune-mediated clearance of the drug from the plasma. Participants who achieved blood Phe $\leq 600 \mu\text{mol/L}$ during the first 24 weeks (Group A) had lower ADA responses compared with participants who did not achieve this blood Phe target until after 24 weeks (Group B). Importantly, all participants but one Group B participant (who discontinued early due to an AE) were able to achieve substantial blood Phe reduction with additional dose titration and longer time on treatment.

Continued dosing in the extension study allowed participants with later treatment response (Group B) to be titrated to higher doses of pegvaliase to overcome the higher rate of antibody-mediated clearance

and achieve reduced blood Phe levels. NABs, capable of inhibiting enzymatic activity of the PAL enzyme [30], were initially detectable only in Group B participants, but eventually developed in all participants during long-term treatment. Participants with stable NAB responses were able to sustain blood Phe reductions likely because the neutralizing response to pegvaliase was a minor component of the overall immune response, with NABs only partially inhibiting enzymatic activity *in vivo*.

Overall, HAEs occurred most frequently in the first 24 weeks of treatment when most subjects were in induction and titration periods of treatment and when the early immune response comprised of PEG IgM, PEG IgG and PAL IgM responses peaked and complement levels were decreasing. The frequency of HAEs decreased during long-term treatment as the immune response matured, PEG IgM and IgG antibodies and PAL IgM responses diminished, and PAL IgG antibodies became most prevalent. The lack of drug-specific IgE detection coupled with the temporal association of HAEs with the peak of antibodies during early treatment and declining complement levels suggests that the predominant mechanism of HAEs observed is type III immune complex-mediated [30, 31]. The primarily PAL IgG antibodies of the mature immune response are likely less able to bind to the drug product, and therefore fix complement than PEG antibodies due to PAL epitope masking by the extensive pegylation on the surface of the drug product. Despite the temporal association with antibody responses and HAEs, antibody positivity rates and mean antibody titers were not predictive of HAEs because of individual variability in immune responses.

Both the 165–205 and PAL-003 studies were open-label and did not include a placebo or control group for comparison. Although participants were instructed to maintain a stable diet, the protocol allowed them to increase dietary Phe at the investigator's discretion in cases of hypophenylalaninemia, and there was an increase in mean protein intake concurrent with maintaining blood Phe $\leq 600 \mu\text{mol/L}$. In addition, the IgM and NAB assays in this study were qualitative (positive/negative), so the potential effects of high and low titers of antibodies (IgM or NAB) could not be quantitatively assessed. Semi-quantitative IgM and NAB titer assays have since been developed for the phase 3 studies of pegvaliase.

While PKU is one of the most studied inborn errors of metabolism, a significant burden of illness and unmet need remain as the majority of adult patients cannot achieve treatment-recommended blood Phe levels. Pegvaliase allowed most patients in this study to achieve blood Phe levels $\leq 600 \mu\text{mol/L}$ demonstrating it has the potential to improve patient care and long-term outcomes. The impact of pegvaliase treatment on neurocognitive outcomes is under investigation in an ongoing phase 3 study.

4.1. Conclusions

With induction, titration, and maintenance dosing regimen, pegvaliase treatment reduced and sustained blood Phe levels in individuals with PKU with a manageable safety profile in most participants. Blood Phe reduction in response to pegvaliase was influenced by dose, treatment duration, and individual immune response to pegvaliase. Despite differences in early treatment response, participants who achieved maintenance dose in the first 24 weeks of pegvaliase treatment (Group A) and those who required additional time and dose titration in the extension study (Group B) achieved meaningful blood Phe reduction with significant natural protein intake. With additional time on treatment and dose titration, later Phe responders (Group B) achieved benefit similar to early Phe responders (Group A); and though dosing was 2–3 times higher in Group B, Group B experienced AE rates that declined and became more similar to participants in Group A in long-term treatment. The results of this study defined the induction and titration dosing strategy currently used in phase 3 clinical studies of pegvaliase treatment for PKU (NCT01819727 and NCT01889862).

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Authorship contributions

CD was the medical monitor. ML performed the statistical analysis. All authors interpreted the results, reviewed early and final drafts of the manuscript, and were fully responsible for the content and editorial decisions related to this manuscript.

Informed consent

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from all participants before being included in the study.

Submission declaration

All authors approved the final version of the manuscript and the decision to publish. This manuscript is not under consideration elsewhere.

Disclosures

BioMarin Pharmaceutical Inc. provided funding for the study and editorial support to develop the publication. RZ, JAT, NS, WBR, and NL are investigators of BioMarin Pharmaceutical Inc. clinical trials and received research support. RZ, JAT, and NL are on the steering committee for the clinical program and have participated in advisory boards of BioMarin. RZ reports grants and consulting fees (BioMarin). WBR has received grants (BioMarin, Genzyme, Shire, Aldeyra Therapeutics). NL reports grants (Aeglea, BioMarin, Genzyme, Horizon, Lumos Pharma, Protalix, Retrophin, Shire, Stealth Therapeutics, Ultragenix), consultant fees (Aeglea, BioMarin, Censa Pharmaceuticals, Dimension Therapeutics,

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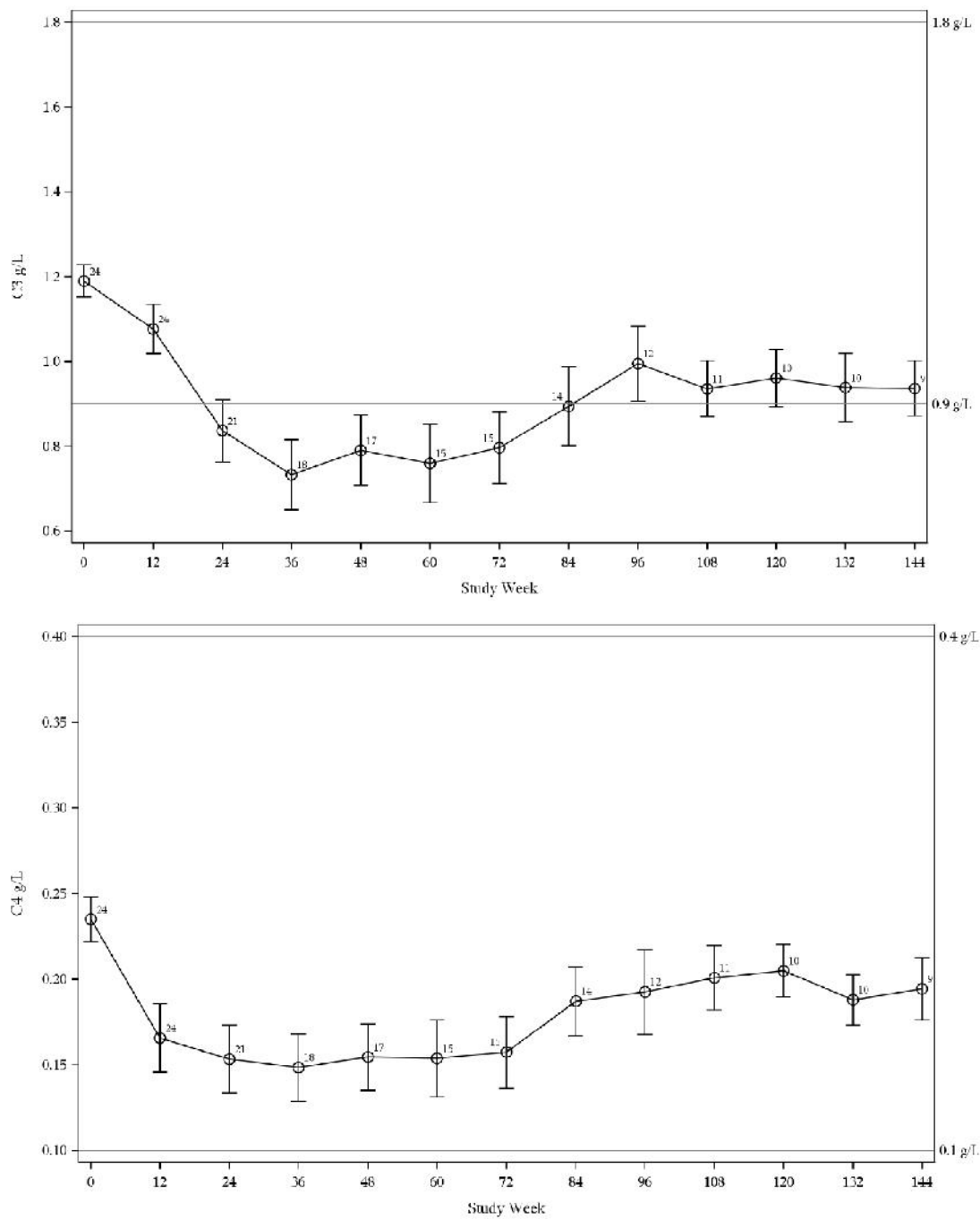
Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ymgme.2018.06.010>.

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Supplementary Figure 1. Mean (SE) Complement Levels Over Time. (A) Complement Component 3. (B) Complement Component 4. Upper and lower limit of normal marked by a horizontal line.