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Pegvaliase for the treatment of phenylketonuria: Results of the phase 2 dose-finding studies with long-term follow-up

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ABSTRACT

Background: Phenylketonuria (PKU) is characterized by a deficiency in phenylalanine hydroxylase (PAH) that may lead to elevated blood phenylalanine (Phe) and significant neurocognitive and neuropsychological co-morbidities. Pegvaliase (PALYNZIQ®, BioMarin Pharmaceutical Inc.) is a PEGylated recombinant *Anabaena variabilis* phenylalanine ammonia lyase (PAL), which converts Phe to trans-cinnamic acid and ammonia, and was approved in May 2018 in the United States and in May 2019 in the European Union for decreasing blood Phe levels in adults with PKU with blood Phe levels > 600 μmol/L. The efficacy and safety of pegvaliase was assessed in two phase 2 dose-finding studies in adults with PKU (PAL-002, NCT00925054, and PAL-004, NCT01212744). Participants completing these studies could enroll in a long-term extension study (PAL-003, NCT00924703).

Methods: Participants in PAL-002 received pegvaliase 0.001, 0.003, 0.01, 0.03, or 0.1 mg/kg weekly for 8 weeks, then continued treatment for a further 8 weeks with dose and/or frequency adjusted to achieve blood Phe concentrations of 60 to 600 μmol/L. Participants in PAL-004 received pegvaliase 0.001 to 0.4 mg/kg 5 days/week for 13 weeks, with modifications made to the starting dose in response to safety and/or efficacy, followed by 3 additional weeks of follow-up assessments. The maximum allowable daily dose in both studies was 1.0 mg/kg/day (5.0 mg/kg/week). Participants who completed any of the phase 2 studies (PAL-002; PAL-004; or a third phase 2 study, 165-205) were eligible to enroll in an open-label, multicenter, long-term extension study (PAL-003, NCT00924703).

Results: Thirty-seven of the 40 enrolled participants completed PAL-002 and 15 of the 16 enrolled participants completed PAL-004. Mean blood Phe at baseline was 1311.0 (standard deviation [SD] 354) μmol/L in PAL-002 and 1482.1 (SD 363.5) μmol/L in PAL-004. Mean blood Phe did not substantially decrease with pegvaliase treatment in PAL-002 (−206.3 [SD 287.1] μmol/L at Week 16) or PAL-004 (−410.8 [SD 653.7] μmol/L at Week 13). In PAL-004, mean blood Phe dropped from baseline by 929.1 μmol/L (SD 691.1) by Week 2; subsequent to dose modifications and interruptions, this early decrease in mean Phe level was not sustained. With increased pegvaliase dose and duration in PAL-003, mean blood Phe levels steadily decreased from baseline, with mean reductions by Week 120 of 68.8% (SD 44.2%) in PAL-002 participants and 75.9% (SD 32.4%) in PAL-004 participants. All participants in PAL-002 and PAL-004 reported ≥1 adverse event (AE), with higher exposure-adjusted event rates in PAL-004. The majority of AEs were mild (87.2% in PAL-002, 86.7% in PAL-004) or

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moderate (12.4% in PAL-002, 13.3% in PAL-004). The most commonly reported AEs in PAL-002 were injection site reaction (50.0% of participants), headache (42.1%), injection site erythema (36.8%), nausea (34.2%), and arthralgia (29.0%), and in PAL-004 were arthralgia (75.0%), headache (62.5%), dizziness (56.3%), injection site erythema (56.3%), and injection site reaction (50.0%).

Conclusions: In two phase 2 dose-finding studies, pegvaliase did not lead to substantial blood Phe reductions. Higher and more frequent pegvaliase dosing in PAL-004 led to a substantial initial drop in blood Phe, but an increase in the number of hypersensitivity AEs and dose reductions or interruptions. With increased dose and duration of treatment in PAL-003, mean blood Phe reduction was substantial and sustained, and the frequency of hypersensitivity AEs decreased and stabilized. Together, these studies led to the development of an induction-titration-maintenance regimen that has been approved for pegvaliase, with patients starting at a low weekly dose that gradually increases in dose and frequency until they achieve a standard non-weight-based daily maintenance dose. This regimen has been tested in a third phase 2 study, as well as in two successful phase 3 studies of pegvaliase.

1. Introduction

Phenylketonuria (PKU) is an autosomal recessive disorder characterized by a deficiency in phenylalanine hydroxylase (PAH), the enzyme that converts phenylalanine (Phe) to tyrosine [1,2]. PAH deficiency leads to elevated blood Phe that is associated with significant neurocognitive and neuropsychological dysfunction [1,2]. The American College of Medical Genetics and Genomics (ACMG) guidelines recommend lifelong treatment of PKU to lower blood Phe to between 120 μmol/L and 360 μmol/L [3]. Current treatment options consist of a Phe-restricted diet and supplementation with Phe-free, amino acid-modified medical foods and specially modified low-protein foods, alone or with sapropterin dihydrochloride (KUVAN®, BioMarin Pharmaceutical Inc., Novato, CA) [3,4]. However, only 20% to 56% of patients with PKU are responsive to sapropterin dihydrochloride [4–6], and many adults and adolescents with PKU still find it difficult to control blood Phe levels long-term, as lifetime adherence to the severely restricted diet is challenging [7]. Patients report not only low palatability of medical foods, but also a fear of stigmatization in social situations, at work, or when traveling when using medical foods [8,9]. Furthermore, the severely restricted diet can also lead to nutritional insufficiencies [3]. Until recently, there were no treatment options for adults with PKU who are unable to keep blood Phe levels controlled long-term and are nonresponsive to sapropterin dihydrochloride.

Pegvaliase (PALYNZIQ®, BioMarin Pharmaceutical Inc.) was approved to reduce blood Phe levels in patients with PKU with blood Phe levels > 600 μmol/L in the United States in May 2018 for adults (aged ≥18 years) and in the European Union in May 2019 for adolescents and adults (aged ≥16 years) [10,11]. Pegvaliase is a recombinant form of the phenylalanine ammonia lyase (PAL) enzyme from *Anabaena variabilis* that has been PEGylated to increase stability and reduce immunogenicity [12,13]. PAL catalyzes the conversion of Phe to ammonia and trans-cinnamic acid, which are then metabolized by the liver and excreted in the urine, respectively [12]. The phase 1 dose-escalation study of pegvaliase in adults with PKU, PAL-001 (NCT00634660), showed that a single 0.1-mg/kg dose decreased blood Phe levels [14].

Here we report on the safety and blood Phe-lowering effects of pegvaliase evaluated in two phase 2 dose-finding studies in adults with PKU (PAL-002, NCT00925054; PAL-004, NCT01212744) and the long-term outcomes of patients from PAL-002 and PAL-004 who continued in an open-label, multicenter, long-term extension study (PAL-003, NCT00924703). The findings of PAL-002 and PAL-004 informed the induction-titration-maintenance dosing schedule tested in a third phase 2 study, 165-205 (NCT01560286) [15], and the phase 3 studies PRISM-1 (NCT01819727) and PRISM-2 (NCT01889862) [16,17]. Participants who completed any of the three phase 2 studies (PAL-002, PAL-004, and 165-205) were eligible to enroll in PAL-003. Longo *et al.* [18] recently reported the long-term results of PAL-003; the outcomes from PAL-003 included in this report are limited to those participants who enrolled from the PAL-002 and PAL-004 studies.

2. Methods

2.1. Study design

PAL-002 and PAL-004 (conducted from September 2009 to July 2012 and from March 2011 to March 2012, respectively) were phase 2, open-label, multisite, dose-finding studies to evaluate the safety, efficacy, and tolerability of multiple subcutaneous doses of pegvaliase in adults with PKU in the United States. The primary objective was to evaluate the effect of various weight-based doses of pegvaliase on blood Phe levels over 16 weeks when given once-weekly (PAL-002) or daily (for 5 days/week, PAL-004). The secondary objective was to evaluate the safety of pegvaliase. Participants who completed PAL-002 or PAL-004 were eligible to enroll into PAL-003, an ongoing, long-term, phase 2 extension study in which the pegvaliase dose could be adjusted to minimize adverse events (AEs) and to achieve or maintain blood Phe concentrations in the range of 60 μmol/L to 600 μmol/L [18]. Participants were instructed to maintain a diet that provided a consistent protein intake throughout PAL-002, PAL-004, and PAL-003.

2.2. Study participants

PAL-002 and PAL-004 included adults with a diagnosis of PKU with a blood Phe concentration of ≥600 μmol/L at the screening visit and an average blood Phe concentration of ≥600 μmol/L for 3 years (PAL-002, not previously enrolled in PAL-001) or 6 months (PAL-004) prior to enrollment. Completion of PAL-001 was also adequate to confer eligibility into PAL-002. To participate in PAL-004, individuals were required to be treatment naive (i.e., could not have had previous treatment with pegvaliase). Individuals were eligible for PAL-002 or PAL-004 if they had not responded to and had discontinued sapropterin dihydrochloride ≥6 months prior to screening. Exclusion criteria included pregnancy or breastfeeding.

2.3. Study drug administration

Participants (N = 40) in PAL-002 received 1 of 5 fixed starting doses of pegvaliase as a subcutaneous injection: 0.001, 0.003, 0.01, 0.03, or 0.1 mg/kg/week for 8 weeks. Participants in PAL-002 were assigned to each fixed-dose cohort sequentially, starting with the lowest dose. Participants were assigned to the next highest dose cohort after the participants in the previous dose cohort had completed Week 3. PAL-002 participants who had previous experience with pegvaliase in the phase 1 study were not assigned to the same dose they had received in the phase 1 study. Participants then continued treatment for another 8 weeks, during which the dose and/or dose frequency could be adjusted to achieve a blood Phe concentration of 60 μmol/L to 600 μmol/L, up to a maximum dose of 5.0 mg/kg/week. Dose modifications were allowed for safety reasons throughout the entire study.

Participants (N = 16) in PAL-004 received pegvaliase as a

subcutaneous injection in doses ranging from 0.001 to 0.4 mg/kg daily for 5 days per week for 13 weeks, followed by 3 additional weeks of follow-up assessments. Groups of 3 participants were enrolled to receive 0.4 mg/kg daily; after 2 weeks, safety and efficacy were assessed and the dose was either continued or decreased. Dose-level adjustments were allowed throughout the study in response to efficacy and/or safety. The maximum allowable daily dose was 1.0 mg/kg/day (5.0 mg/kg/week).

Participants from PAL-002 (n = 33) or PAL-004 (n = 15) who entered PAL-003 could continue pegvaliase following the dosing regimen from the parent study or could receive a higher dose as determined by a medical monitor and the primary investigator on a per-patient basis. In PAL-003, the dose level and frequency could be adjusted throughout the study for each participant to achieve a blood Phe concentration of between 60 and 600 μmol/L or for safety reasons. Doses were similar to those tested in the parent studies and ranged between 0.001 mg/kg/week and 5 mg/kg/week, administered over up to 7 days a week. Following an October 2014 protocol amendment, the maximum weekly dose was 375 mg/week, regardless of participant weight.

2.4. Assessments

The primary efficacy endpoint was the change from baseline through Week 16 in blood Phe levels in PAL-002 and from baseline through Week 13 in PAL-004. Blood Phe concentration was measured at the screening visit and weekly for 16 weeks thereafter. Participants who continued to PAL-003 continued with assessments every 4 or 12 weeks. Safety was assessed based on vital signs, physical examination, AEs, and laboratory tests. AEs were assessed at every weekly clinic visit, and data on AE incidence, exposure-adjusted event rate, and severity grade (National Cancer Institute-Common Terminology Criteria for Adverse Events [NCI-CTCAE] mild, moderate, severe, life-threatening, or death [19]) were collected. AEs were coded by preferred term based on the Medical Dictionary for Regulatory Activities (MedDRA) version 16.0 [20].

2.5. Statistical analysis

The efficacy and safety analyses included all participants in the PAL-002 and PAL-004 studies and available follow-up data from participants who continued treatment in PAL-003 as of October 27, 2016. Descriptive statistics were reported for continuous variables and categorical variables separately for the PAL-002 and PAL-004 studies and for the participants who continued to PAL-003. Weekly pegvaliase dose, calculated as the sum of the doses in the 7 days prior to assessment, was used to describe treatment drug exposure for this analysis. Blood Phe concentration, change from baseline in blood Phe concentration, and achievement of blood Phe thresholds (≤120, ≤360, and ≤600 μmol/L) were evaluated. Baseline was defined as the last blood Phe measurement prior to the first dose of pegvaliase in any study. AEs were summarized by preferred term and severity. AEs of special interest included injection site reactions, arthralgia, hypersensitivity AEs (HAEs), and acute systemic hypersensitivity events. HAEs were defined by the hypersensitivity standardized MedDRA query (SMQ) that included the preferred terms arthralgia, arthritis, eye inflammation, eye irritation, eye pain, joint stiffness, joint swelling, pyrexia, vision blurred, and polyarthritis, and the broad anaphylactic reaction SMQ. After completion of PAL-002 and PAL-004, all acute systemic hypersensitivity events were reviewed by an independent allergist/immunologist to identify events consistent with anaphylaxis per criteria from the National Institute of Allergy and Infectious Diseases/Food Allergy and Anaphylaxis Foundation (NIAID/FAAN) [21]. Identified events were further classified as nonsevere or severe according to Brown's severity criteria [22]. Exposure-adjusted AE rates were calculated to allow comparisons between the shorter PAL-002 and PAL-004 studies and the longer PAL-003 study.

3. Results

3.1. Participant disposition and characteristics

Forty participants enrolled in PAL-002 at 10 US sites and were included in the efficacy and safety analyses; of these, 37 participants completed the study and 3 discontinued the study. One participant discontinued due to an AE (mild skin reaction that resolved on treatment with oral/topical antihistamine and topical corticosteroids), 1 was lost to follow-up, and 1 withdrew consent. An additional participant discontinued treatment with pegvaliase due to an AE (mild arthralgia that resolved after treatment with aspirin, ibuprofen, chondroitin, glucosamine, and diclofenac/misoprostol), but remained in the study for assessments. Thirty-three of the 37 participants who completed PAL-002 continued on to the PAL-003 long-term extension study. Three participants chose not to participate in PAL-003, and 1 participant, who discontinued treatment due to mild arthralgia but remained in PAL-002 for assessments, was not eligible. During PAL-003, 14 participants discontinued: 1 due to an AE (severe arthralgia and severe peripheral neuropathy that resolved with treatment), 3 based on physician decision, 7 who withdrew consent, and 3 who were lost to follow-up.

A total of 16 participants from 7 different sites enrolled in PAL-004 and were included in the safety and efficacy analyses. Of these, 15 completed the study and enrolled in the PAL-003 long-term extension study; 1 participant withdrew consent after experiencing moderate angioedema that was considered a severe AE (SAE, see description below). During PAL-003, 2 patients discontinued: 1 based on physician decision and 1 who withdrew consent.

Mean ages at enrollment were similar for PAL-002 (26.1 [standard deviation (SD) 6.4] years) and PAL-004 (32.2 [SD 8.3] years) (Table 1). Mean baseline blood Phe levels were also similar in PAL-002 (1311 [SD 354] μmol/L) and PAL-004 (1482 [SD 363.5] μmol/L). The demographics of the participants who continued to PAL-003 were similar to those in the parent studies (data not shown).

3.2. Pegvaliase exposure

Per each study's protocol, dose and dosing frequency varied as individuals progressed through PAL-002 and PAL-004, and as they continued to PAL-003. Mean weekly pegvaliase exposure increased from 11.4 (SD 11.9) mg/week in PAL-002 to 178.2 (SD 142.3) mg/week in PAL-003, and from 39.6 (SD 33.9) mg/week in PAL-004 to 168.7 (SD 95.6) mg/week in PAL-003 (Table 2). The average weekly doses of pegvaliase in PAL-004 were higher compared with those in PAL-002 (Fig. 1). After entering PAL-003, the pegvaliase dose increased, with an average weekly dose of 142.2 (SD 109.1) mg. Pegvaliase dose was relatively stable by Week 120 of treatment and remained stable throughout the follow-up period in PAL-003.

3.3. Blood Phe levels

Mean blood Phe decreased by 206.3 (SD 287.1) μmol/L, to 1088.5 (SD 367.7) μmol/L at Week 16 in PAL-002 (Fig. 1A). In PAL-004, mean blood Phe decreased by 410.8 (SD 653.7) μmol/L, to 1045.1 (SD 663.94) μmol/L at Week 13 (Fig. 1B). In PAL-004, a reduction in mean blood Phe was evident at Week 1, and a decrease from baseline of 929.1 (SD 691.1) μmol/L, the largest drop in the study, was evident by Week 2. When pegvaliase dosing was interrupted or reduced in many participants at Week 2 subsequent to HAEs, mean blood Phe increased to near baseline levels and then remained at this level through the end of the study.

Although no substantial change was seen in mean blood Phe levels

Table 1
Participant baseline characteristics.

	PAL-002 (N = 40)		PAL-004 (N = 16)	
	Parent Study (n = 40)	Long-term Extension (PAL-003) (n = 33)	Parent Study (n = 16)	Long-term Extension (PAL-003) (n = 15)
Age at enrollment (years)				
Mean (SD)	26.1 (6.4)	26.1 (6.6)	32.2 (8.3)	32.2 (8.6)
Min, Max	16, 43	16, 43	18, 50	18, 50
≥18 years, n (%)	38 (95)	31 (94)	16 (100)	15 (100)
< 18 years, n (%)	2 (5)	2 (6.1)	0	0
Female, n (%)	20 (50)	16 (48.5)	13 (81.3)	12 (80)
BMI (kg/m^2)				
Mean (SD)	29.1 (7.6)[a]	29.6 (8.2)[b]	29.5 (9.3)	29.6 (9.6)
Min, Max	19.1, 56.2[a]	19, 56.2	17.2, 47.1	17.2, 47.1
< 30, n (%)	24 (60)[a]	18 (58)	10 (62.5)	9 (60)
≥30, n (%)	13 (32.5)[a]	13 (42)	6 (37.5)	6 (40)
Blood Phe concentration[c] (μmol/L)				
Mean (SD)	1311 (354)	1369.1 (302.2)	1482.1 (363.5)	1455.9 (360.2)
Min, Max	249[d], 1878	741, 1878	968, 2214	968, 2214

BMI, body mass index; Max, maximum; Min, minimum; SD, standard deviation.
[a] n = 37.
[b] n = 31.
[c] Baseline blood Phe values are mean values before entering PAL-002 or PAL-004.
[d] One participant who entered PAL-002 from the phase 1 study had a baseline Phe value of 249 μmol/L.

from baseline to the end of the PAL-002 and PAL-004 studies, when the dose and duration of pegvaliase treatment were increased in participants who continued to PAL-003, mean blood Phe levels steadily decreased over time (Fig. 1A and 1B). In participants who enrolled in PAL-003 from PAL-002, mean blood Phe decreased to 428.2 (SD 606.2) μmol/L at Week 120 (n = 25), a 68.8% (SD 44.2%) decrease from baseline. In participants who enrolled in PAL-003 from PAL-004, mean blood Phe decreased to 350.4 (SD 503.9) μmol/L at Week 120 (n = 12), a 75.9% (SD 32.4%) decrease from baseline.

In PAL-002, 4 (10%) participants achieved a blood Phe level ≤600 μmol/L, including 1 (2.5%) participant who achieved a blood Phe level ≤120 μmol/L at least once during the 16-week study. In PAL-004, 4 (25%) participants achieved a blood Phe level ≤600 μmol/L, including 3 (18.7%) participants who achieved a blood Phe level ≤120 μmol/L at least once during the 13-week study. After enrolling in PAL-003, during which dose adjustments were allowed, the number of participants from PAL-002 and PAL-004 who achieved a blood Phe level ≤120 μmol/L increased to 27 (81.8%) and 13 (87%), respectively.

3.4. Adverse events

All participants in both parent studies reported at least 1 AE (Table 3). The exposure-adjusted event rate was higher in PAL-004 than in PAL-002 (119.6 vs 43.9 events per person-year). Overall, the AE rate decreased with continued treatment in the PAL-003 long-term extension study, dropping to 17.9 and 21.0 events per person-year in participants from PAL-002 and PAL-004, respectively. The most commonly reported AEs in PAL-002 were injection site reaction (50.0% of participants), headache (42.1%), injection site erythema (36.8%), nausea (34.2%), and arthralgia (29.0%), with exposure-adjusted event rates of 6.1, 2.1, 2.5, 1.8, and 2.2 events per person-year, respectively (Supplemental Table 1). The most commonly reported AEs in PAL-004 were arthralgia (75.0% of participants), headache (62.5%), dizziness (56.3%), injection site erythema (56.3%), and injection site reaction (50.0%), with exposure-adjusted event rates of 10.2, 9.7, 4.0, 6.5, and 11.1 events per person-year, respectively.

The majority of AEs in both parent studies were mild (87.2% in PAL-002, 86.7% in PAL-004) or moderate (12.4% in PAL-002, 13.3% in PAL-

Table 2
Pegvaliase exposure.

	PAL-002 (N = 40)		PAL-004 (N = 16)	
	Parent Study (n = 40)	Long-term Extension (PAL-003) (n = 33)	Parent Study (n = 16)	Long-term Extension (PAL-003) (n = 15)
Average dose (mg/week)				
Mean (SD)	11.4 (11.9)	178.2 (142.3)	39.6 (33.9)	168.7 (95.6)
Median	7.9	144.7	28.8	169
Min, Max	0.9, 58.7	25.6, 750.8	6.5, 129.2	36.4, 351.6
Total treatment duration (weeks)				
Mean (SD)	14.4 (2.0)	202.4 (113.5)	11.5 (2.9)	180.9 (82.0)
Median	15.1	211.3	12.0	213.6
Min, Max	7.1, 15.9	4.1, 354.3	0.7, 12.7	42.4, 268.4
Last weekly dose received (mg/week)				
Mean (SD)	40.0 (43.0)	128.4 (101.1)	56.7 (46.6)	142.2 (109.1)
Median	23.8	86.0	47.6	120.0
Min, Max	0.8, 214.6	13.3, 360.0	2.19, 175.0	4.0, 431.0

All data from phase 2 studies are included.
Max, maximum; Min, minimum; SD, standard deviation.

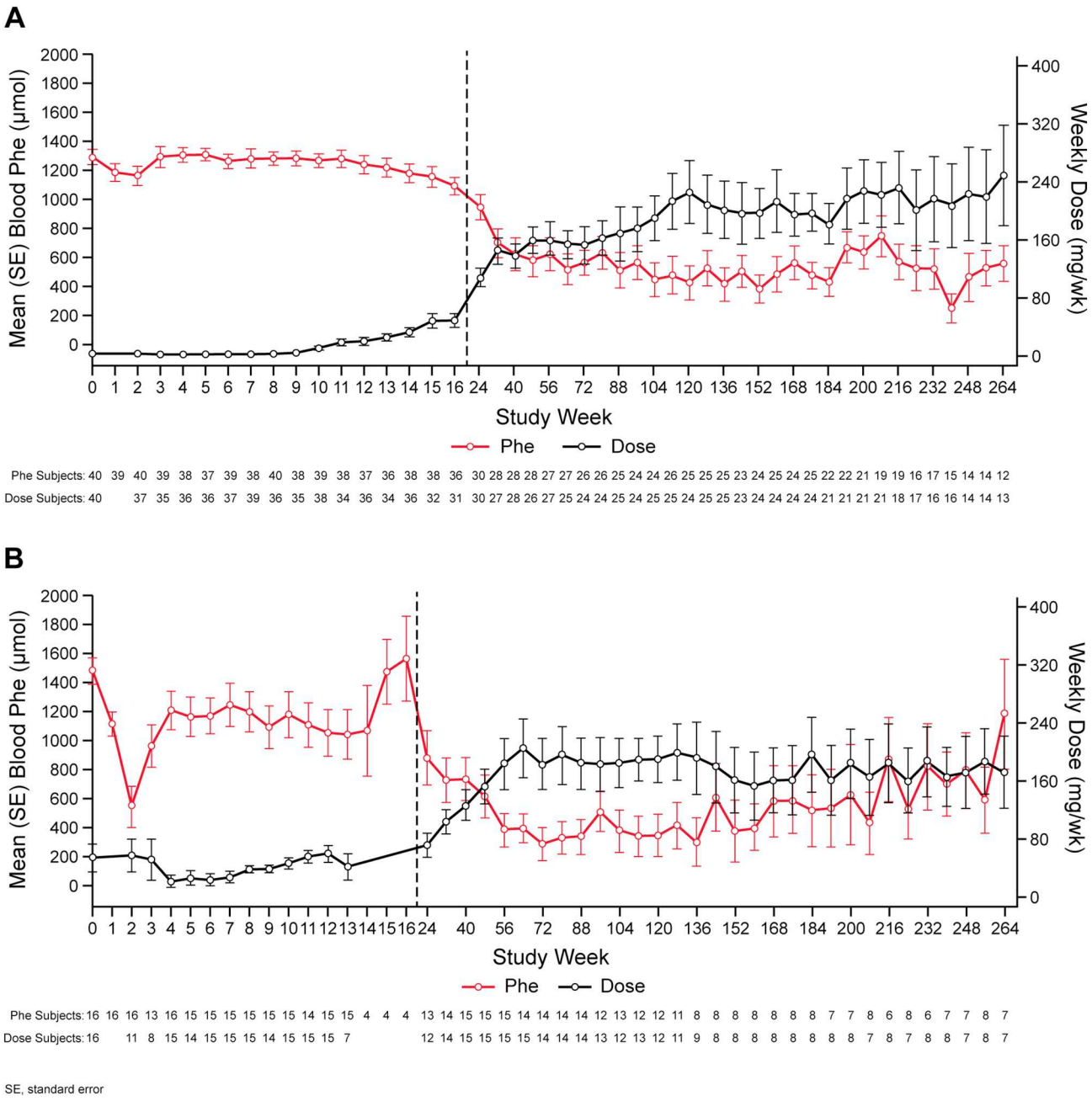

Fig. 1. Mean blood Phe concentration and pegvaliase dose over time in (A) PAL-002 and (B) PAL-004 continuing through PAL-003. Data are presented as mean (SE). Dotted line indicates transition of participants from the PAL-002 or PAL-004 studies to the PAL-003 study. Sample size reflects the participants with data available at the indicated timepoint and who had reached the timepoint at the time of the data cut; the study was ongoing at the time of this analysis.

004). Two participants in PAL-002 reported SAEs (hypersensitivity and dehydration); both events resolved and neither event led to withdrawal from treatment or the study. One SAE of moderate angioedema was reported in PAL-004 and led to discontinuation of the study drug. Following medical review performed by the sponsor, the SAE was assessed to be clinically consistent with anaphylaxis based on NIAID/FAAN criteria [21] (see details in section 3.6). The majority of AEs resolved without dose interruption or reduction (90% in PAL-002, 84% in PAL-004). AEs led to a greater number of treatment interruptions in PAL-004 compared with PAL-002 (15.6 vs 2.8 events per person-year, respectively). No deaths occurred in the PAL-002 or PAL-004 studies.

3.5. Hypersensitivity adverse events

The majority of participants in both studies experienced an HAE, and injection site reaction was the most common HAE in PAL-002 (n = 30, 75%) and PAL-004 (n = 16, 100%). Most HAEs were mild or moderate in severity. As described above, 1 participant in PAL-002 developed a serious HAE that resolved and did not lead to study discontinuation; an additional 2 participants in PAL-002 developed mild HAEs that led to pegvaliase discontinuation (n = 1 skin reaction, n = 1 arthralgia). The participant who experienced the skin reaction was treated with topical and oral diphenhydramine and topical

Table 3
AEs reported by participant incidence (*n*, %) and event rate (events/person-year).

	PAL-002				PAL-004			
	Parent Study (*N* = 40)		Long-term Extension (PAL-003) (*N* = 33)		Parent Study (*N* = 16)		Long-term Extension (PAL-003) (*N* = 15)	
	Total exposure = 11.1 person-years		Total exposure = 128.4 person-years		Total exposure = 3.5 person-years		Total exposure = 52.2 person-years	
	Incidence, *n* (%)	Event rate	Incidence, *n* (%)	Event rate	Incidence, *n* (%)	Event rate	Incidence, *n* (%)	Event rate
Any AE (exposure, person-years)	40	43.9	33	17.9	16	119.6	15	21.0
AE causing pegvaliase interruption	7 (17.5)	2.8	10 (30.3)	0.4	8 (50.0)	15.6	5 (33.3)	0.2
AE causing pegvaliase reduction	5 (12.5)	1.6	3 (9.1)	0.1	3 (18.8)	3.1	2 (13.3)	0.1
AE causing pegvaliase discontinuation	2 (5.0)	0.2	2 (6.1)	0.1	1 (6.3)	0.3	0 (0)	0
AE causing study discontinuation	1 (2.5)	0.1	1 (3.0)	0.02	0 (0)	0	0 (0)	0
Any SAE	2 (5.0)	0.2	8 (24.2)	0.1	1 (6.3)	0.3	0 (0)	0
SAE causing pegvaliase discontinuation	0 (0)	0	1 (3.0)	0.02	1 (6.3)	0.3	0 (0)	0
SAE causing study discontinuation	0 (0)	0	1 (3.0)	0.02	0 (0)	0	0 (0)	0
Death	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0
AEs of special interest								
HAE	31 (77.5)	8.8	30 (90.9)	3.5	16 (100.0)	31.0	15 (100.0)	3.5
Acute systemic hypersensitivity event of anaphylaxis	1 (2.5)	0.1	0 (0)	0	1 (6.3)	0.3	0 (0)	0
Injection site reaction	30 (75.0)	14.0	28 (84.9)	4.2	16 (100.0)	30.1	15 (100.0)	3.5
Arthralgia	11 (27.5)	2.2	19 (57.6)	0.8	12 (75.0)	10.2	6 (40.0)	0.4

Event rate was calculated as the total number of events divided by person-years of exposure. Incidence rates counted participants who reported more than 1 AE within a preferred term only once.
AE, adverse event; HAE, hypersensitivity adverse event; SAE, severe adverse event.

hydrocortisone cream and discontinued the study. One participant in PAL-004 discontinued pegvaliase due to an HAE (angioedema SAE consistent with acute systemic hypersensitivity), but continued in the study.

The exposure-adjusted rate of HAEs was higher in PAL-004 (31.0 events per person-year) compared with PAL-002 (8.8 events per person-year). The highest frequency of HAEs in PAL-004 occurred at Week 2 (n = 40 events, 33.9% of all HAEs reported during study), by which time the majority of participants had reported the onset of an HAE (n = 12), angioedema (n = 4), or an acute systemic hypersensitivity event categorized as anaphylaxis per NIAID/FAAN criteria (n = 1; see section 3.6). Although HAEs continued to be reported for the remainder of PAL-004, the frequency of the reactions was lower than its peak at Week 2. The HAE rate decreased as participants continued treatment in the PAL-003 long-term extension study, decreasing to 3.5 events per person-year among participants from PAL-002 and PAL-004.

3.6. Acute systemic hypersensitivity events

One participant in PAL-002 (2.5%) and 1 participant in PAL-004 (6.3%) experienced an acute systemic hypersensitivity event that was confirmed as anaphylaxis by an independent allergist/immunologist after study completion. In PAL-002, the participant experienced rash, chest tightness, and shortness of breath approximately 17 to 27 hours after injection on Day 70 of the study (Dose 11); symptoms were mild and did not meet Brown's severity criteria. The pegvaliase dose remained unchanged and the event was treated with oral antihistamines, resolved within 1 day, and did not lead to study discontinuation.

In PAL-004, 1 participant experienced angioedema within 2 minutes of the injection on Day 79 of the study (Dose 55; she had experienced arthralgia, generalized rash, and joint stiffness with prior injections). Symptoms were graded as moderate and the event was considered to be an SAE (see section 3.4). The participant also experienced symptoms of nausea, vomiting, flushing of the upper extremities, abdominal pain, and diarrhea. Pegvaliase was withdrawn and symptoms resolved after treatment with oral and intramuscular diphenhydramine and intravenous hydration. The participant discontinued from the study drug due to the event.

None of the events required epinephrine use and/or emergency department visit, and drug-specific immunoglobulin E antibodies were not detected at or near the time of either event.

4. Discussion

In two open-label, multisite, prospective, phase 2, dose-finding studies of pegvaliase in adults with PKU (PAL-002 and PAL-004), pegvaliase treatment did not result in substantial reductions in blood Phe levels over 16 weeks and 13 weeks, respectively. For tolerability reasons, the first phase 2 study, PAL-002, used a conservative pegvaliase dosing schedule with low starting doses. Thus, it is not surprising that the majority of participants did not achieve the target blood Phe level of ≤600 μmol/L in that study. Although higher and more frequent pegvaliase dosing in PAL-004 led to a substantial initial drop in blood Phe, it also led to an increased number of HAEs and subsequent dose reductions or interruptions in treatment. The per-study dose interruption at 2 weeks in PAL-004 led to an increase in mean blood Phe to near baseline levels, which remained elevated throughout the remainder of the study.

The majority of participants in both parent studies entered into and continued treatment throughout the long-term extension study, with a combined pegvaliase exposure of 195.2 person-years as of the October 2016 cut-off date. With increased dose and duration of treatment, these participants experienced substantial and sustained blood Phe reduction, with a decrease in AE frequency over time.

As understanding of pegvaliase evolved during the phase 2 trials, the dosing of pegvaliase also evolved from a weight-based dosing approach to standard doses given in an induction-titration-maintenance regimen. This regimen, which starts with low weekly dosing that is gradually increased to higher daily maintenance dosing, was tested in the 165-205 phase 2 study and confirmed in two successful phase 3 studies (PRISM-1 and PRISM-2) [15–17]. In 165-205, after a 4- to 8-week low-dose induction period, pegvaliase was titrated up in dose and

frequency over a minimum of 4 weeks until blood Phe levels ≤600 μmol/L were achieved; participants then continued on the maintenance dose [15]. Approximately half of the participants in 165-205 achieved the maintenance dose within 24 weeks; the majority of participants continued treatment and achieved their maintenance dose by 48 weeks. As in PAL-002 and PAL-004, the exposure-adjusted AE rates were higher earlier in the 165-205 study and decreased with continued treatment. The phase 3 studies subsequently demonstrated that the pegvaliase induction-titration-maintenance regimen resulted in a substantial and sustained decrease in blood Phe levels consistent with the lower targets recommended by ACMG guidelines [3]. Over a 24-month period, 61% of participants reached blood Phe concentrations ≤360 μmol/L and 51% of participants reached blood Phe levels of ≤120 μmol/L [17]. Most AEs were mild or moderate and decreased in frequency with continued treatment.

As dose-finding studies, the PAL-002 and PAL-004 studies have some notable limitations, including an open-label design, small study populations, and no comparator or placebo groups. In addition, while participants were directed to maintain a diet consistent in protein intake, dietary intake data were not regularly monitored or collected in the PAL-002 study. Finally, the population-level analyses reported here do not capture blood Phe level fluctuations (as levels were captured weekly and then monthly) or the effects of treatment on blood Phe levels in individual participants. Nevertheless, the PAL-002 and PAL-004 study findings directly informed the development of a more tolerable induction-titration-maintenance regimen that was shown to be effective in reducing blood Phe levels in the 165-205 phase 2 study and PRISM phase 3 studies, which enrolled nearly 300 participants with PKU [15,17].

Based on the collective findings of the phase 2 and phase 3 studies, pegvaliase addresses an unmet need as a therapy for adults with PKU to effectively maintain reduced blood Phe levels, allowing many patients to achieve target blood Phe levels recommended by current ACMG guidelines [3], with a manageable safety profile for most patients.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ymgme.2020.06.006.

Declaration of Competing Interest

BKB received consulting fees and/or honoraria (Aeglea, Agios, Alexion, BioMarin, Chiesi, Denali, Horizon, JCR Pharma, Moderna, Shire [Takeda], Sanofi Genzyme, Ultragenyx) and conducted contracted research (BioMarin, Homology Medicines, Shire [Takeda], Sangamo, Ultragenyx). NL has received consulting fees (Aeglea, BioMarin, Censa Pharmaceuticals, Dimension Therapeutics, Genzyme/Sanofi, Hemoshear, Horizon, Lumos Pharma, Moderna, Mitobridge, Pfizer, Retrophin, Stealth Therapeutics) and conducted contracted research (Aeglea, BioMarin, Genzyme/Sanofi, Horizon, Lumos Pharma, Protalix, Retrophin, Shire, Stealth Therapeutics, Ultragenyx). JV has received research funding from BioMarin. DKG has received consulting fees (Dermelix) and conducted contracted research (BioMarin, Edimer Pharmaceuticals, Sanofi Genzyme, Shire). COH has received consulting fees, speaker fees, and travel and research support from BioMarin. JT has received consulting fees and research support from BioMarin. CD, ML, KLau, OR, and KLarimore are employees of BioMarin.

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