



Research paper

Association of immune response with efficacy and safety outcomes in adults with phenylketonuria administered pegvaliase in phase 3 clinical trials



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ABSTRACT

Background: This study assessed the immunogenicity of pegvaliase (recombinant *Anabaena variabilis* phenylalanine [Phe] ammonia lyase [PAL] conjugated with polyethylene glycol [PEG]) treatment in adults with phenylketonuria (PKU) and its impact on safety and efficacy.

Methods: Immunogenicity was assessed during induction, upward titration, and maintenance dosing regimens in adults with PKU ($n = 261$). Total antidrug antibodies (ADA), neutralizing antibodies, immunoglobulin (Ig) M and IgG antibodies against PAL and PEG, IgG and IgM circulating immune complex (CIC) levels, complement components 3 and 4 (C3/C4), plasma Phe, and safety were assessed at baseline and throughout the study. Pegvaliase-specific IgE levels were measured in patients after hypersensitivity adverse events (HAE).

Findings: All patients developed ADA against PAL, peaking by 6 months and then stabilizing. Most developed transient antibody responses against PEG, peaking by 3 months, then returning to baseline by 9 months. Binding of ADA to pegvaliase led to CIC formation and complement activation, which were highest during early treatment. Blood Phe decreased over time as CIC levels and complement activation declined and pegvaliase dosage increased. HAEs were most frequent during early treatment and declined over time. No patient with acute systemic hypersensitivity events tested positive for pegvaliase-specific IgE near the time of the event. Laboratory evidence was consistent with immune complex-mediated type III hypersensitivity. No evidence of pegvaliase-associated IC-mediated end organ damage was noted.

Interpretation: Despite a universal ADA response post-pegvaliase administration, adult patients with PKU achieved substantial and sustained blood Phe reductions with a manageable safety profile.

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1. Introduction

Phenylketonuria (PKU; OMIM 261600), also known as phenylalanine hydroxylase (PAH) deficiency, is a rare, autosomal recessive disease resulting in high concentrations of blood phenylalanine (Phe). Elevated blood Phe in adults is associated with executive dysfunction and significant behavioral and psychiatric problems, which lead to a

negative impact on patient quality of life [1–4]. A recent survey of patients with PKU reported that only 23% of adult patients [5] were able to maintain blood Phe within the therapeutic range of 120 to 360 $\mu\text{mol/L}$ [6].

Treatment for PKU includes severe restriction of dietary Phe, supplemented with Phe-free or low-Phe amino acid-modified medical foods, alone or in combination with sapropterin dihydrochloride (KUVAN[®], BioMarin Pharmaceutical Inc., Novato, CA). However, for most adults with PKU, these treatments are insufficient to manage their chronic disease due to long-term adherence issues or inadequate Phe-lowering effects [1,5,7–9].

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Research in context

Evidence before this study

Phenylketonuria (PKU), an autosomal recessive disease caused by phenylalanine hydroxylase deficiency, results in high blood phenylalanine (Phe) levels leading to neuropsychiatric and neurocognitive symptoms. Pegvaliase is a PEGylated, bacterially produced, phenylalanine ammonia lyase (PAL) enzyme substitution therapy that lowers blood Phe by converting Phe to trans-cinnamic acid and ammonia. Protein therapeutics have been shown to induce antidrug antibodies (ADA), which can impact clinical safety and efficacy. Development of anti-polyethylene glycol (PEG) antibodies following treatment with PEGylated therapeutics has been reported, but the nature of these antibodies and their clinical consequences are not well established.

Added value of this study

This is the first time that the immunogenicity of a chronically administered, bacterially derived, PEGylated enzyme therapeutic and its impact on safety and efficacy have been characterized. During early treatment (≤ 6 months after initiation), patients developed an immune response composed of anti-PEG antibodies and anti-PAL immunoglobulin (Ig) M. During late treatment (> 6 months after initiation), anti-PEG antibodies reduced to baseline levels and the immune response was mainly anti-PAL IgG and IgM. Circulating immune complexes (CICs) and complement activation were highest in early treatment and decreased as the anti-PEG antibodies decreased, suggesting that the PEGylation was effective in shielding binding of PAL-specific antibodies to the drug and forming CICs. Hypersensitivity adverse events were most frequent in early treatment and decreased as CICs and complement activation lessened, but required slow titration of the drug to efficacious levels. Blood Phe concentrations decreased over time as the immune response matured and pegvaliase dosage increased.

Implications of all the available evidence

Patients treated with pegvaliase developed ADA responses that matured to a clinical non responsive state over time, allowing substantial and sustained blood Phe reduction to be achieved with a manageable safety profile for most patients.

Pegvaliase (Palynziq™, BioMarin Pharmaceutical Inc., Novato, CA) is a bacterially derived (recombinant *Anabaena variabilis* [rAv]) phenylalanine ammonia lyase (PAL) conjugated with polyethylene glycol (PEG) and produced in *Escherichia coli*. PEGylation of the PAL enzyme reduces immune recognition and improves pharmacodynamic stability. Pegvaliase, an enzyme substitution therapy, metabolizes Phe to trans-cinnamic acid and ammonia. Based on results from phase 3 studies showing substantial and sustained reductions in mean blood Phe and long-term improvements in symptoms of inattention and mood, pegvaliase is approved in the United States to treat adult patients with PKU and is the first bacterially derived therapeutic protein approved for the treatment of a chronic disease [4,7,10].

Biologics, including enzyme replacement therapies (ERTs), are used for a range of disease treatments. Many ERTs induce antidrug antibodies (ADAs) that result in adverse events (AEs) and reduced efficacy, and/or impact pharmacokinetics [11,12]. AEs may be due to the formation of drug/ADA circulating immune complex (CIC) intermediates with downstream effects [13]. Neutralizing antibody (NAb) formation can inhibit

enzymatic activity and efficacy [14,15]. Given that pegvaliase contains bacterially derived PAL, development of ADAs after treatment with pegvaliase is expected. Further, an increasing number of reports document development of anti-PEG antibodies following treatment with PEGylated therapeutics [16–18]. Anti-PEG antibodies have been associated with reduced efficacy and hypersensitivity with some therapeutics, but it is unclear why only some PEGylated therapeutics induce a clinically relevant anti-PEG antibody response [16,18]. Therefore, the objective of this study was to comprehensively characterize the immunogenicity profile after pegvaliase treatment, including anti-PEG and anti-PAL antibody responses and CIC and complement levels, and assess the impact of that response on safety and efficacy.

2. Materials and methods

The methodology has been previously described for the phase 3 PRISM-1 and PRISM-2 studies (ClinicalTrials.gov Identifiers: NCT01819727, NCT01889862) [4]. Patients in the phase 3 trials self-administered subcutaneous pegvaliase using an induction, titration, and maintenance dosing schedule. Patients were randomized in PRISM-1 to titrate to a maintenance dose of 20 mg/day or 40 mg/day of pegvaliase. In PRISM-2, dosing of 5 to 60 mg/day of pegvaliase was allowed [4]. Details on the structural and biochemical characteristics of rAvPAL (accession number: 3CZO) have previously been reported [19]. Baseline blood Phe and immunogenicity data are provided (Supplemental Table 1). Informed consent was obtained from each participant and the PRISM studies were conducted in accordance with the Declaration of Helsinki. Prior to initiating the study, the investigator at each study site obtained written confirmation that the institutional review board (IRB) was properly constituted and compliant with International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use and Good Clinical Practice requirements and applicable laws and local regulations. A complete list of the IRBs is provided in the Supplemental Text. An independent data monitoring committee governed the conduct of the phase 3 trials.

2.1. Assessments

Blood Phe (measured in plasma), immunogenicity (measured in serum), and safety assessments were conducted at baseline and at least monthly during the induction, titration, and maintenance periods until 1 year on treatment, at which point assessments were conducted bimonthly. Established CLIA/CAP methods were used to measure Phe, and ADA assays were validated to assess serum positivity and titers for anti-pegvaliase total antibodies (TAb), PAL immunoglobulin (Ig) M, PAL IgG, PEG IgM, PEG IgG, and antibodies that inhibit pegvaliase enzyme activity (NAb). IgG and IgM CICs were measured at baseline and at approximately 12, 36, 60, 84, and 108 weeks after first dose. For acute systemic hypersensitivity events (see “Safety measurements”), a clinic visit was required for measurement of anti-pegvaliase IgE and other clinical laboratory parameters (high-sensitivity C-reactive protein, complement components 3 and 4 [C3/C4] antigens, and tryptase).

2.2. Safety measurements

Safety was monitored using clinical laboratory tests (chemistry, hematology, and urinalysis), vital signs, physical examinations, electrocardiograms, and AEs (coded by MedDRA preferred terms [20]). AEs suggestive of immune complex (IC) formation were assessed; these included arthralgia/arthritis, generalized skin reaction lasting ≥ 14 days, urticaria lasting ≥ 14 days, lymphadenopathy lasting ≥ 14 days, serum sickness-like reaction, serious infections, and ischemic heart disease. Search strategies for laboratory data identified abnormalities that could be reflective of IC disease. Hypersensitivity adverse events (HAEs) were identified using broad modified hypersensitivity Standardized MedDRA Queries (SMQ), which included additional preferred

term AEs of arthralgia, arthritis, eye inflammation, eye irritation, eye pain, joint stiffness, joint swelling, pyrexia, blurred vision and polyarthritis, and the broad algorithmic anaphylactic reaction SMQ. The anaphylactic reaction SMQ, hypersensitivity SMQ, and MedDRA preferred terms were used to identify all AEs that could be component manifestations of acute systemic hypersensitivity events and/or reported as anaphylactic reactions. An allergist/immunologist independent of the clinical site and sponsor reviewed all potential episodes of acute systemic hypersensitivity events to identify the events consistent with clinical criteria of anaphylaxis defined by the National Institute of Allergy and Infectious Diseases/Food Allergy and Anaphylaxis Network [21] and Brown's severe criteria (ie, hypoxia, hypotension, or neurologic compromise) [22].

2.3. Assays

Semi-quantitative direct-format electrochemiluminescent titer assays were used to measure PAL-specific IgM, PAL-specific IgG, PEG-specific IgM, PEG-specific IgG, and anti-pegvaliase TAB titers in serum. Pegvaliase NAb titers were measured using a semi-quantitative hybrid ligand-binding liquid chromatography/tandem mass spectrometry method. Pegvaliase-specific IgE was measured using an ImmunoCAP assay that was modified to include a step whereby IgG and IgM were depleted from serum before IgE assay. Complement-activating C3s containing IgG and complement C3d were measured in serum using the MicroVue CIC-Raji Cell Replacement (also referred to as CIC-C3d EIA) assay (Quidel Corporation, San Diego, CA). The MicroVue assay was modified to measure CIC containing IgM and C3d. Decreases in C3 and C4 levels were monitored in the serum as biomarkers of complement activation. Phe concentrations were measured in deproteinized plasma using ion exchange chromatography coupled with ninhydrin derivatization. Details of each assay method are provided (see Supplemental Text).

2.4. Statistical analyses

The analysis population included all patients who first initiated pegvaliase in PRISM-1 and included all data collected in PRISM-1 and PRISM-2 as of September 23, 2016. Baseline values were defined as the last available measurement prior to the first administration of pegvaliase in PRISM-1 (ie, pegvaliase-naïve baseline). Blood Phe data were summarized at monthly intervals relative to baseline. The safety analyses included data on the incidence, exposure-adjusted event rate, and severity based on Common Terminology Criteria for Adverse Events grade (grade 1, mild; grade 2, moderate; grade 3, severe but not immediately life-threatening; grade 4, life-threatening; grade 5, death). An exposure-adjusted event rate analysis was performed to control for differences in duration of study participation.

Analysis of the association of CIC levels with blood Phe reduction was performed by dividing patients into quartiles based on mean IgG-CIC or IgM-CIC change from baseline over the entire treatment duration. To evaluate the relationship between antibody titers and HAEs, patients were divided into quartiles based on their mean antibody titer during early (≤ 6 months) or late (> 6 months) treatment phases plotted against the number of HAEs. To evaluate associations between CIC concentrations and exposure-adjusted AE rates, patients were divided into quartiles based on their mean CIC change from baseline level calculated from all available results. Mean percent change in blood Phe was calculated for each quartile at each evaluated time point. Patients with complement reduction $< 25\%$ and $\geq 25\%$ at Year 1 from baseline were compared to evaluate the association of complement reduction with AEs or labs of interest.

3. Results

Two hundred and sixty-one patients enrolled in PRISM-1, as described previously [4]. Mean \pm standard deviation (SD) blood

concentration of Phe at baseline was $1232.7 \pm 386.36 \mu\text{mol/L}$, indicating poor control.

3.1. Development of a biphasic immune response against pegvaliase

All patients developed TAB responses against pegvaliase (Supplemental Table 2), comprising subpopulations of treatment-induced ADA to PAL and treatment-induced or treatment-boosted ADA to PEG. The antibody response occurred in a biphasic manner; the response in early treatment (≤ 6 months after treatment initiation) was primarily composed of anti-PEG IgM, anti-PEG IgG, and anti-PAL IgM, whereas the response in late treatment (> 6 months) was composed of anti-PAL IgM and anti-PAL IgG (Fig. 1a and b).

Anti-PEG antibody titers peaked at 1 to 3 months after treatment initiation and then returned to baseline in most patients by 6 to 9 months. The incidence of anti-PEG IgG and anti-PEG IgM at baseline was 52.3% and 45.0% (Supplemental Table 2), respectively, resulting from previous exposures to PEG, which is within the range reported in the literature [23–25]. The presence of anti-PEG antibodies at baseline was not associated with an increased number of hypersensitivity events (Supplemental Table 3).

Anti-PAL IgM and IgG titers peaked at 3 and 6 months, respectively, and remained stable through long-term treatment. The majority of patients developed a low-titer neutralizing ADA (NAb) response, which remained stable through long-term treatment (mean NAb titer at 6 months: 415 [$n = 192$]; 1 year: 608 [$n = 144$]; 2 years: 661 [$n = 91$]; Supplemental Table 2).

Mean CIC levels were highest during early treatment and then decreased, corresponding with the change in anti-PEG responses over time. Peak IgG-containing CIC levels were associated with declining C3 and C4 levels, indicating complement activation (Fig. 1c). Overall, there was a high degree of consistency in the pattern of IgG CIC and IgM CIC development over time, suggesting that CICs comprised both IgM and IgG and can be detected with either assay method.

Antibody titers and IgG CIC levels either decreased (ie, anti-PEG antibodies) or stabilized (ie, anti-PAL antibodies) over time and did not increase after dose increases (Fig. 1c, Supplemental Fig. 1).

3.2. Immunogenicity and efficacy

The immune response to pegvaliase was associated with blood Phe reduction. During early treatment, anti-PEG IgG, anti-PEG IgM, anti-PAL IgM, and CIC levels were highest, C3/C4 levels were lowest, and plasma Phe concentrations were high (blood Phe [mean \pm SD] = $922.5 \pm 525.1 \mu\text{mol/L}$, $n = 237$ at 3 months). In late treatment, anti-PEG IgG, anti-PEG IgM, CIC, and C3/C4 levels returned toward baseline values, pegvaliase dose was adjusted to individual blood Phe response, and blood Phe concentrations decreased (blood Phe [mean \pm SD] = $1232.7 \pm 386.4 \mu\text{mol/L}$ at baseline; $564.5 \pm 531.2 \mu\text{mol/L}$ at 12 months; $311.4 \pm 426.6 \mu\text{mol/L}$ at 24 months).

As shown in Fig. 2, the magnitude of the NAb immune response was inversely associated with the degree of blood Phe reduction. In early treatment, patients in the lowest antibody titer quartile (Q1) experienced the largest reductions in blood Phe. Following stabilization of antibody titers and dose adjustment, mean blood Phe concentrations continued to decrease, including in patients within higher antibody titer quartiles (Q2, Q3, and Q4). A similar trend was observed across all antibody types (non-neutralizing, neutralizing), analytes (anti-PAL, anti-PEG, TAB), and isotypes (IgM, IgG). A related pattern was also observed between the magnitude of the CIC levels and blood Phe reduction. Quartile analysis of IgG CIC levels (Fig. 3) indicated that patients with less change from baseline in IgG CIC had more rapid mean blood Phe reduction, although substantial blood Phe reduction was observed in all IgG CIC quartiles.

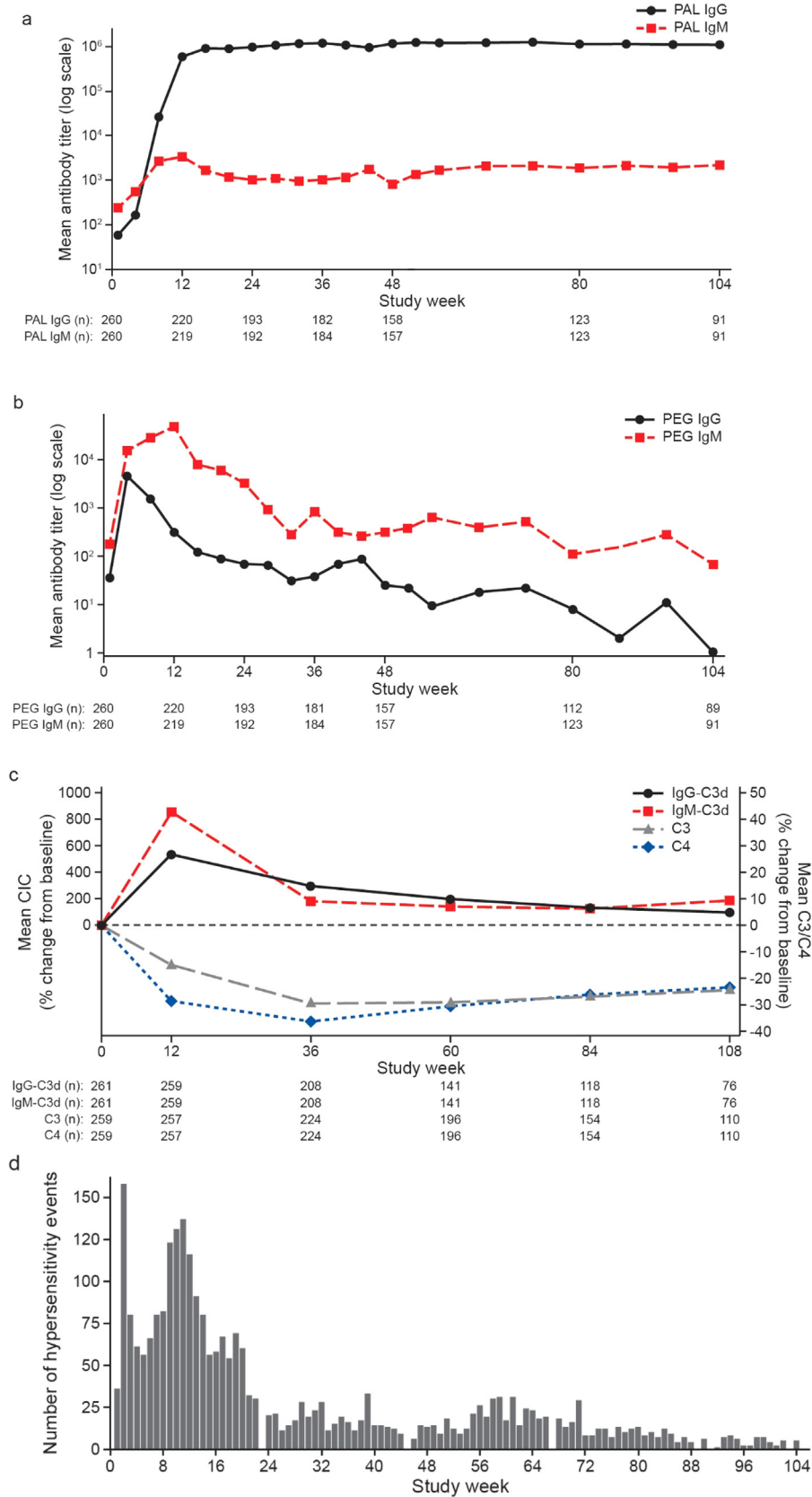


Fig. 1. Anti-pegvaliase ADA, CIC, and C3/C4 response (a) PAL IgG and PAL IgM titers, (b) PEG IgG and PEG IgM titers, (c) percent change in CIC and C3/C4 levels from baseline, (d) hypersensitivity adverse events. ADA, antidrug antibody; C3/C4 complement components 3 and 4; CIC, circulating immune complex; IgG, immunoglobulin G; IgM, immunoglobulin M; PAL, phenylalanine ammonia lyase; PEG, polyethylene glycol.

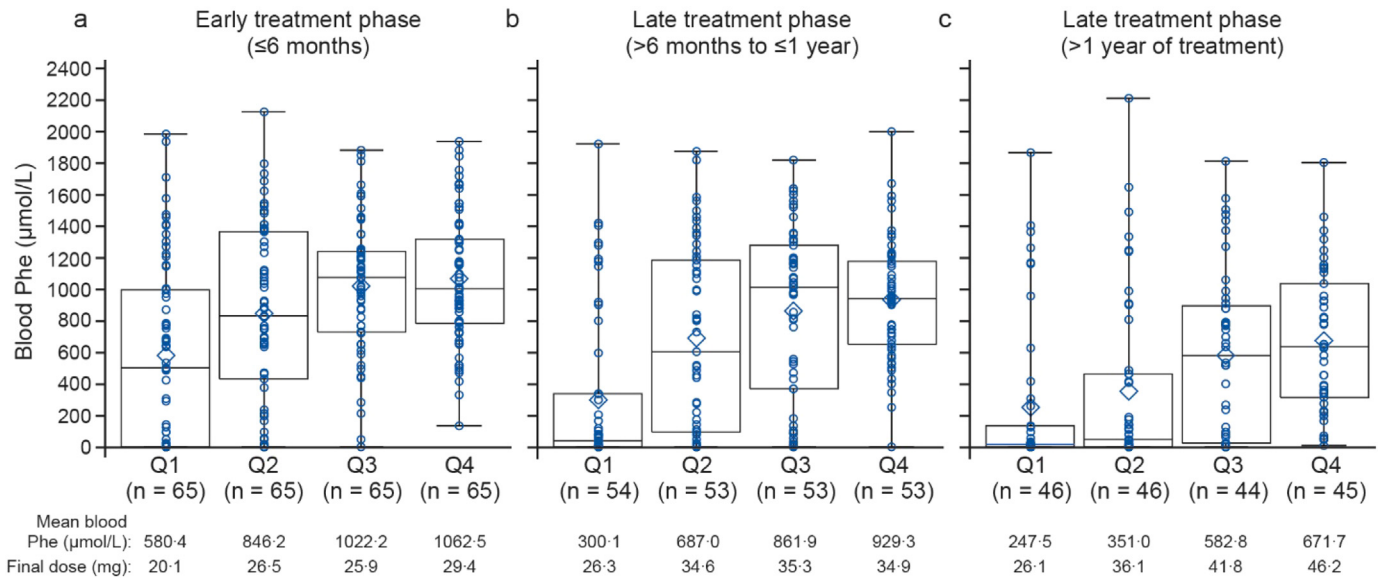


Fig. 2. NAb quartiles and blood Phe. Subject data were divided into quartiles based on mean post-baseline antibody titer (a) ≤6 months after start of treatment, (b) >6 months to ≤1 year after start of treatment, and (c) >1 year after start of treatment. Observed blood Phe was defined as the last available post-baseline blood Phe within each time interval. NAb, neutralizing antibody; Phe, phenylalanine; Q, quartile.

3.3. Immunogenicity and safety

Almost all patients (93.5%) experienced HAEs. HAEs occurred most frequently in early treatment (15.6 events/patient-year) when anti-PEG IgM, anti-PEG IgG, and anti-PAL IgM responses were peaking, CIC levels were high, and C3/C4 levels were low (Fig. 1d). The frequency of HAEs declined over time (HAE rate after 6 months = 4.0 events/patient-year), as the anti-PAL antibody response stabilized and the anti-PEG response, CIC, and complement activation decreased over time.

A total of 17 events in 12 patients (4.6%) were adjudicated by an independent allergist as acute systemic hypersensitivity events, with

15 of these events occurring in the first year of treatment. No patient required intubation or vasopressor treatment to treat acute hypersensitivity, and all the events resolved without sequelae. Pegvaliase-specific IgE was not detected in serum from patients at or near the time of the event. Six patients discontinued from the study after these events while 6 continued dosing; of those, 2 had additional events that did not occur directly after restarting pegvaliase [26].

Antibody titers were not associated with HAEs during any treatment phase, as demonstrated in Supplemental Fig. 2 using TAB titers as an example. There was significant overlap of HAEs in patients from all TAB quartiles; thus, antibody titers were not predictive of development of HAEs.

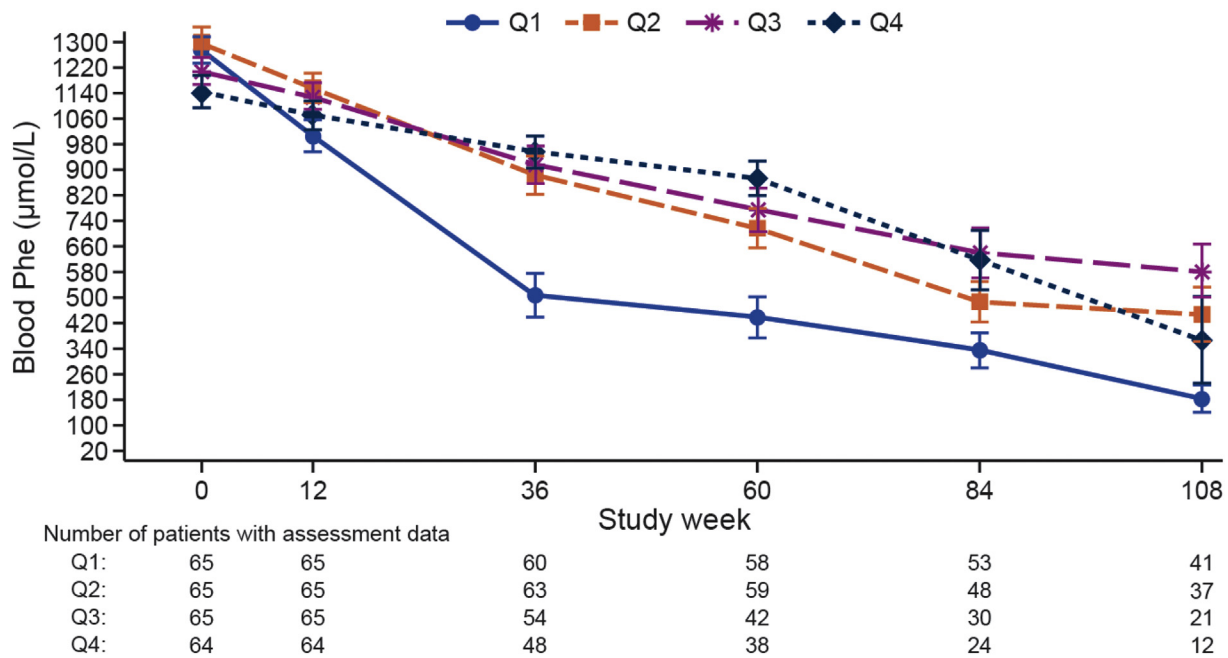


Fig. 3. IgG CIC quartiles and blood Phe. Subject data were divided into quartiles based on mean CIC change from baseline level calculated from all available results, which was plotted with mean blood Phe concentration for each quartile at each evaluated time point. The averages of all values within each interval are plotted. Due to differences in the duration of time on study for individual patients and discontinuation rates, there are a limited number of patients with data at time points after week 84. CIC, circulating immune complex; IgG, immunoglobulin G; Phe, phenylalanine; Q, quartile.

Table 1

Adverse events by IgG circulating immune complex change from baseline quartiles (intent-to-treat population, N = 261).

	Quartile 1 (n = 65)	Quartile 2 (n = 65)	Quartile 3 (n = 65)	Quartile 4 (n = 64)	Total (n = 259)
IgG CIC change from baseline quartile range (min–max)	(–22.76–10.85)	(10.98–21.11)	(21.54–35.20)	(35.46–242.25)	
Total treatment exposure, person-years ^a	125.5	125.3	84.6	65.8	401.2
Event rate, per person-year (total number of events)					
Adverse events	22.58 (2833)	28.74 (3603)	34.37 (2909)	45.10 (2967)	30.69 (12312)
Serious adverse events	0.05 (6)	0.18 (22)	0.11 (9)	0.35 (23)	0.15 (60)
Hypersensitivity adverse events	5.83 (731)	7.67 (961)	7.46 (631)	9.41 (619)	7.33 (2942)
Acute systemic hypersensitivity events of anaphylaxis	0	0.02 (2)	0.04 (3)	0.18 (12)	0.04 (17)
Arthralgia/arthritis	2.45 (308)	2.39 (300)	2.71 (229)	4.09 (269)	2.76 (1106)
Generalized skin reaction ≥14 days duration	0.52 (65)	0.42 (53)	0.39 (33)	0.30 (20)	0.43 (171)
Urticaria ≥14 days duration	0.02 (3)	0.02 (3)	0.02 (2)	0.03 (2)	0.02 (10)
Lymphadenopathy ≥14 days duration	0.09 (11)	0.12 (15)	0.11 (9)	0.29 (19)	0.13 (54)
Serum sickness-like reaction	0.02 (2)	0.02 (2)	0.02 (2)	0.02 (1)	0.02 (7)
Infection and infestations ≥30 days duration	0.16 (20)	0.23 (29)	0.25 (21)	0.23 (15)	0.21 (85)
Infection serious adverse events	0.01 (1)	0.03 (4)	0	0.02 (1)	6 (0.01)
Ischemic heart disease	0	0	0	0	0

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 patients (for each patient, time from the first dose to the last dose administered across all studies in which the patient was enrolled). Intervals of missing doses that were > 28 consecutive days were excluded from the calculation of treatment duration. CIC, circulating immune complex; IgG, immunoglobulin G.

As shown in Table 1, AEs, serious AEs (SAEs), HAEs, acute systemic hypersensitivity events, arthralgia/arthritis, and lymphadenopathy lasting ≥14 days were highest in patients with the largest IgG CIC increase from baseline. Results for IgM CIC were similar to IgG CIC (Supplemental Table 4). The trend observed with SAEs was driven by acute systemic hypersensitivity events.

Overall, exposure-adjusted AE rates were similar for patients with <25% or > 25% C3 reduction from baseline at 1 year with the exception of acute systemic hypersensitivity events, which occurred at a higher rate in patients with ≥25% C3 reduction (Table 2). Results were similar for C3 and C4.

No specific antibody titer value, CIC level, or complement level was predictive of AEs, including acute systemic hypersensitivity events. Wide individual variability was observed in antibody titers, CIC levels, and complement levels between patients with and without acute systemic hypersensitivity events (Fig. 4).

No clinical observations or laboratory abnormalities suggestive of pegvaliase-associated IC-mediated end-organ damage, including renal failure, central nervous system manifestations (such as cerebrovascular accidents or transient ischemic attacks), myocardial ischemic events, or hemolytic anemia events were observed in any pegvaliase-treated patients.

4. Discussion

Historically, reports characterizing immunogenicity against biologics have been limited to a description of ADA incidence. However,

many of the immune-mediated clinical effects attributed to ADAs require the formation of a drug/ADA IC intermediate, which is seldom measured and can have a variety of downstream effects. In this study, we report on the immunogenicity of pegvaliase using an extensive monitoring strategy, including measurement of antibody titers of multiple isotypes and specificities, assessment of enzymatic neutralization, measurement of IgM and IgG IC, and analyses of biomarkers of complement activation. These findings lead to a clear understanding of the immunogenicity of pegvaliase and its impact on efficacy and safety of the drug, enabling the design of a dosing paradigm aimed to achieve maximum efficacy while minimizing AEs.

Patients treated daily with a subcutaneous injection of pegvaliase developed antibodies against PAL and PEG. The sustained anti-PAL response is consistent with a typical CD4-dependent antibody response induced against a foreign protein [27]. The transient nature of the anti-PEG response may result from a low-affinity, CD4-independent antibody response typically observed against non-protein repetitive antigens [13]. CD4-independent antibody responses to PEG have been reported for both nonclinical and clinical studies. In nearly all animal studies, anti-PEG antibody responses were largely IgM and were transient [28,29].

Two of 13 approved PEGylated biotherapeutics, pegaspargase and pegloticase, induce anti-PEG antibodies associated with loss of efficacy and increased risks for hypersensitivity reactions [16]. With both, the anti-PEG antibody responses were sustained and transitioned from a response predominantly IgM to an IgG isotype. This is in contrast to the transient and predominantly IgM and low-titer IgG responses to PEG

Table 2

Adverse events by C3 reduction from baseline at year 1 (intent-to-treat population, N = 233).

Percent reduction in C3 from baseline at year 1	<25% (n = 67)	≥25% (n = 98)	≥25% and < 50% (n = 59)	≥50% (n = 39)
Total treatment exposure, person-years ^a	120.2	188.4	115.7	72.7
Event rate, per person-year (total number of events)				
Any adverse event	31.3 (3760)	29.5 (5562)	26.0 (3005)	35.2 (2557)
Any serious adverse event	0.1 (12)	0.1 (26)	0.1 (16)	0.1 (10)
Any hypersensitivity adverse event	7.1 (857)	7.2 (1363)	7.2 (834)	7.3 (529)
Acute systemic hypersensitivity event of anaphylaxis	0.01 (1)	0.06 (12)	0.05 (6)	0.08 (6)
Arthralgia/arthritis	2.9 (351)	2.6 (490)	2.3 (262)	3.1 (228)
Generalized skin reaction ≥14 days duration	0.6 (68)	0.4 (67)	0.4 (48)	0.3 (19)
Persistent urticaria ≥ 14 days duration	0.02 (2)	0.03 (5)	0.03 (3)	0.03 (2)

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 patients (for each patient, time from the first dose to the last dose administered across all studies in which the patient was enrolled). Intervals of missing doses that were > 28 consecutive days were excluded from the calculation of treatment duration. C3, complement component 3.

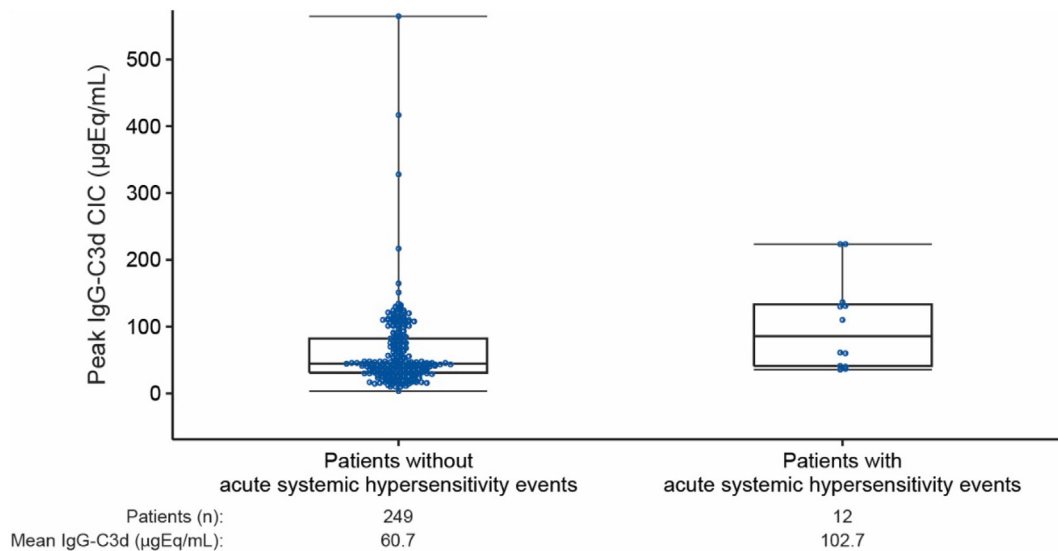


Fig. 4. Peak IgG-C3d CIC concentrations in patients with and without acute systemic hypersensitivity events. There was considerable overlap in IgG CIC levels for patients with and without acute systemic hypersensitivity events, and patients with the highest IgG CIC levels did not experience acute systemic hypersensitivity events. C3d, complement component 3d; CIC, circulating immune complex; IgG, immunoglobulin G.

observed with pegvaliase, perhaps attributable to the induction, titration, and daily maintenance dosing regimen leading to desensitization over time. Anti-PEG antibodies in healthy individuals have been reported due to exposure to PEG-containing compounds in cosmetics, pharmaceuticals, and processed food products, but the presence of these antibodies has not been associated with any pathology. A subset of patients in the PRISM trials enrolled with pre-existing anti-PEG IgG and/or anti-PEG IgM, consistent with the baseline positivity rates reported in the literature [24,25,27]. Presence of anti-PEG antibodies at baseline did not impact pegvaliase safety.

The data presented herein suggest the change in immune response over time influences the dosing necessary to achieve efficacy. High levels of IgM observed during early treatment (≤ 6 months after treatment initiation) are an efficient activator of the classical complement pathway. In contrast, the immune response in late treatment (> 6 months after treatment initiation) is composed predominately of anti-PAL IgG antibodies, which are less likely to bind to the drug product and fix complement due to the masking of PAL epitopes by extensive PEGylation on the surface of the drug. Fig. 5 illustrates our model that CIC levels are highest in early treatment despite sustained anti-PAL ADA levels.

The time course of ADA maturation, IC formation, and complement activation drove the need for an induction/titration dosing schedule necessary to achieve efficacious doses in later treatment while minimizing the occurrence of HAEs during early treatment. The suggested dosing schedule includes a low starting dose of 2.5 mg/week for 4 weeks, followed by a gradual titration over ≥ 5 weeks up to 20 mg/day and a

delayed escalation from 20 to 40 mg/day. The maintenance dose of 20 or 40 mg/day is individualized based on patient tolerability and blood Phe concentrations. For further details on the dosing schedule, please refer to the Palynziq prescribing information [10]. The overlap in the range of antibody titers between the different dose groups makes it difficult to associate a specific antibody titer with a specific dose required to achieve Phe reduction. For this reason, monitoring of blood Phe is recommended for managing patients to ensure the lowest dose is used to achieve reduction in blood Phe.

Data from this study demonstrated that there was an inverse association between the magnitude of the ADA response and the degree of blood Phe reduction. Nevertheless, patients with the highest ADA titers could still achieve a meaningful Phe response once the immune response matured and their dose increased with long-term treatment. The mechanisms by which ADA may impact pegvaliase efficacy include immune-mediated plasma clearance of the drug via the reticuloendothelial system and reduction of enzyme activity following NAB binding. In this study, patients with a wide range of NAB responses were able to attain sustained reductions in blood Phe, suggesting that the neutralizing response to pegvaliase was a minor component of the overall immune response and only partially inhibited *in vivo* enzymatic activity (Fig. 2). Overall, no specific threshold of antibody titer, isotype, or specificity, including NAB, was predictive of the degree of reduction or dose needed to achieve substantial reduction in blood Phe.

In this study, no specific CIC level was predictive of the occurrence of an acute systemic hypersensitivity event despite an association between the magnitude of CIC change from baseline and frequency of

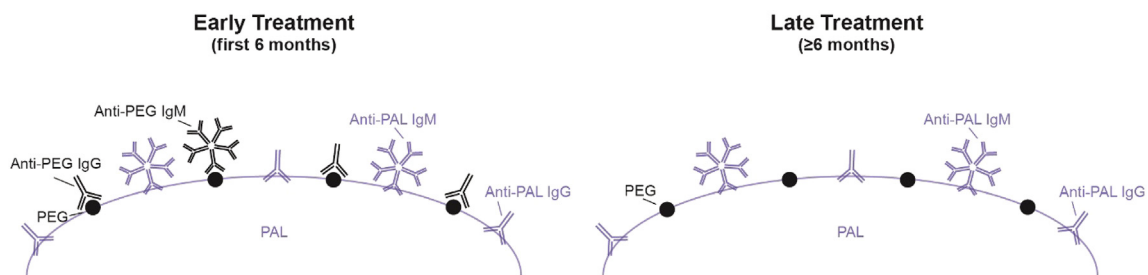


Fig. 5. Illustration of the changing CIC landscape in early vs. late treatment. Early in treatment (0–6 months), CICs contain anti-PEG IgG, anti-PEG IgM, anti-PAL IgG and anti-PAL IgM. Later in treatment (> 6 months), there are fewer CICs formed by anti-PAL IgG and anti-PAL IgM only. CIC, circulating immune complex; IgG, immunoglobulin G; IgM, immunoglobulin M; PAL, phenylalanine ammonia lyase; PEG, polyethylene glycol.

acute systemic hypersensitivity events. Reasons for this may be multifactorial, including the methods used to detect CICs, which detected all CICs rather than those that are antigen-specific, contribution of other mechanisms such as IgG-mediated hypersensitivity reactions via binding to Fc gamma receptors, and presence of other unidentified cofactors that might increase the risk of hypersensitivity in some patients. Although pegvaliase has potential for IC-mediated toxicity, a review of the safety data thus far has shown no evidence of pegvaliase-associated end-organ damage due to IC deposition with 401 patient-years of exposure, including 20 patients with >3 years of exposure.

In summary, these results support the efficacy of pegvaliase for the treatment of adults with PKU, with a manageable safety profile for most participants. Pegvaliase has been shown to have improved safety and efficacy when introduced slowly with incremental dose increases to allow the immune system to stabilize. The risk of AEs is highest in early treatment during induction/titration dosing, when the immune response is immature and patients are receiving low, sub-therapeutic doses. In late treatment, the immune response matures and stabilizes, and the occurrence of HAEs decreases due to a reduction in IC formation and complement activation, despite dose increases that allow the majority of patients to achieve substantial and durable blood Phe efficacy.

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Declaration of interests

SG, KL, RB, JO, KL, ZG, ML, OR, SJZ, HHW, and BS are employees and shareholders of BioMarin Pharmaceutical Inc.

COH reports personal fees and other from BioMarin Pharmaceutical Inc. during the conduct of the study; personal fees from Synlogic, Rubius Therapeutics, Inc., and Pfizer Inc.; and grants from StrideBio, Inc., outside the submitted work.

GS and JPA report personal fees from BioMarin Pharmaceutical Inc. during the conduct of the study.

VK reports grants from BioMarin Pharmaceutical Inc. during the conduct of the study.

Author contributions

SG, KL, COH, GS, RB, JPA, VK, JO, KL, ZG, ML, OR, SJZ, HHW, and BS contributed to critical data interpretation, intellectual content, review, and approval of this manuscript. COH was a primary investigator of the PRISM clinical trials. VK and JPA analyzed the data. VK performed laboratory analyses. All authors read and approved the final version of this manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ebiom.2018.10.038>.

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