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.

Keywords: Enzyme replacement therapy, Antidrug antibody, Circulating immune complex, Hypersensitivity, Phenylalanine

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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 μmol/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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enzyomatic 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 rVA PAL (accession number: 3CZ0) 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 IgG, PEG-specific IgM, 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. 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 ≥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 ± standard deviation (SD) blood concentration of Phe at baseline was 1232.7 ± 386.36 μmol/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 IgC 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 ± SD] = 922.5 ± 525.1 μmol/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 ± SD] = 1232.7 ± 386-4 μmol/L at baseline; 564.5 ± 531.2 μmol/L at 12 months; 311.4 ± 426.6 μmol/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 with 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.
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. 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.

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.
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

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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
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 co-factors 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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