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Phase 1 Trial of Subcutaneous rAvPAL-PEG in Subjects with Phenylketonuria

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Abstract

Objective—Phenylketonuria is an inherited disease caused by impaired activity of phenylalanine hydroxylase, the enzyme that converts phenylalanine to tyrosine, leading to accumulation of phenylalanine and subsequent neurocognitive dysfunction. A phenylalanine-restricted diet initiated early in life can ameliorate the toxic effects of phenylalanine. However, the diet is onerous and compliance is extremely difficult. Phenylalanine ammonia lyase (PAL) is a prokaryotic enzyme that converts phenylalanine to ammonia and trans-cinnamic acid. This Phase 1, multicenter clinical trial evaluated the safety, tolerability, pharmacokinetics and efficacy of rAvPAL-PEG (recombinant *Anabaena variabilis* PAL produced in *E. coli* conjugated with polyethylene glycol [PEG] to reduce immunogenicity) in reducing phenylalanine levels in subjects with phenylketonuria.

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Methods—Single subcutaneous injections of rAvPAL-PEG in escalating doses (0.001, 0.003, 0.01, 0.03, and 0.1 mg/kg) were administered to 25 adults with phenylketonuria recruited from those attending metabolic clinics in North America whose blood phenylalanine concentrations were 600 µmol/L.

Results—The most frequently reported adverse events were injection-site reactions and dizziness. Reactions were self-limited without sequelae. During the trial, two subjects had adverse reactions to intramuscular (IM) medroxyprogesterone acetate, a drug containing polyethylene glycol as an excipient. Three subjects developed a generalized skin rash at the highest rAvPAL-PEG dose (0.1 mg/kg). Drug levels peaked ~5 days after the injection. Treatment was effective in reducing blood phenylalanine in all five subjects receiving the highest dose (0.1 mg/kg, mean percent change of -58 from baseline), with a nadir ~6 days after injection and inverse correlation between drug and phenylalanine concentrations in plasma. Phenylalanine concentrations returned to near-baseline levels ~20 days after the single injection.

Conclusions—Subcutaneous administration of rAvPAL-PEG in a single dose of up to 0.1 mg/kg is safe and well tolerated in subjects with phenylketonuria. At the highest dose tested, rAvPAL-PEG reduced blood phenylalanine concentrations. (NCT00925054 in clinicaltrials.gov)

Introduction

Phenylketonuria (PKU) is an autosomal recessive disease caused by a deficiency of the enzyme phenylalanine hydroxylase (PAH).¹ PAH deficiency results in abnormally elevated concentrations of phenylalanine (Phe) that can affect brain function, causing intellectual disability, microcephaly, delayed speech, seizures, and behavioral abnormalities.¹ Phenylketonuria has an overall incidence of 1:15000 births², with an incidence of 1:10000 in Europe and East Asia¹, 1:2600 in Turkey³, 1:4500 in Ireland⁴ and lower frequency in Africa and Japan^{1, 5}. There are more than 10000 people with PKU in the USA alone, with about 9000 of them born after initiation of newborn screening (<http://www.npkua.org/index.php/pku-facts>). Implementation of a Phe-restricted diet including low-Phe medical formulas and dietary restriction of all protein-containing foods in infancy significantly reduces blood Phe concentrations and prevents severe intellectual disability^{1, 3}. Current recommendations for management of PKU involve lifetime adherence to the Phe-restricted diet to maintain Phe concentrations in recommended ranges of 120-360 µmol/L for children under 12 years of age, 120-600 µmol/L for individuals 12-18 years and 120-900 for subjects >18 years⁶ as well as sapropterin dihydrochloride (sapropterin, Kuvan®) as an adjunct to diet for those individuals who are responsive to this treatment. Compliance with this restrictive diet becomes increasingly more difficult for older children, adolescents, and adults,^{7, 8} and lack of adherence may result in nutritional deficiencies due to the limitations of natural protein sources in addition to inadequate control of plasma Phe concentrations.^{8, 9} The resulting elevation of Phe concentrations has been associated with impairment of executive function and behavioral problems.¹⁰⁻¹⁴ Although sapropterin, a cofactor of PAH, increases residual enzyme activity and reduces Phe concentrations in a subset of subjects with PKU, for many subjects diet remains the only method of therapy.

Phenylalanine ammonia lyase (PAL) is a non-mammalian enzyme that converts Phe to ammonia and trans-cinnamic acid. The recombinant molecule, rAvPAL-PEG (PEGylated

recombinant phenylalanine ammonia lyase) is a genetically modified form of the *Anabaena variabilis* PAL produced in *E. coli*, and subsequently PEGylated to reduce immunogenicity. rAvPAL-PEG reduces blood Phe concentrations in animal models of PKU. In the murine PKU model BTBR^{Pah^{enu2}} (ENU2), a mouse deficient in PAH activity,¹⁵ weekly subcutaneous (SC) administration of 80 mg/kg of rAvPAL-PEG for longer than three months lowered blood Phe concentration from baseline concentrations of 1000–2000 µmol/L to < 200 µmol/L. The objective of this study was to determine whether rAvPAL-PEG given subcutaneously could safely reduce phenylalanine levels in individuals with PKU. This novel therapy could help all patients with PKU in maintaining phenylalanine levels within the therapeutic range, preventing or ameliorating potential behavioral problems or deficits in executive function.

Materials and Methods

Study Design

The study was a Phase 1, multicenter, open-label dose escalation study to assess the safety and biologic activity of a single subcutaneous dose of rAvPAL-PEG (0.001, 0.003, 0.01, 0.03 and 0.1 mg/kg) in subjects with PKU. The dose of rAvPAL-PEG was increased progressively in each group of five subjects until the final dose (0.1 mg/kg). Each subject received a single rAvPAL-PEG injection and was then followed for a total of 43 days (six weeks) with regular visits and laboratory tests. A study period of 6 weeks after drug injection was selected to verify that the drug disappeared from blood and that there was reversal of any therapeutic effect.

The institutional review boards of all the participating institutions approved the study protocol. All procedures were performed in accordance with the *Guideline for Good Clinical Practice* as issued by the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. Each subject gave written informed consent to participate.

Study Subjects

Subjects with PKU 18 years of age or older with blood Phe of ≥ 600 µmol/L at screening and averaging ≥ 600 µmol/L over the previous three years before the study were recruited at one of seven metabolic clinics in the United States. All subjects had a diagnosis of classic phenylketonuria (phenylalanine levels at diagnosis >1200 µmol/L) and were identified among those attending metabolic clinics with a history of poor compliance with dietary treatment. Subjects continued their diet with no significant modification during the four weeks preceding and the six weeks after the administration of rAvPAL-PEG. No formal dietary analysis was performed. Subjects were excluded due to hepatic (alanine amino transferase > 2 times upper limit of normal) or renal (serum creatinine > 1.5 mg/dL) dysfunction, pregnancy or potential pregnancy, use of nicotine or drugs of abuse or use of any investigational products within 30 days prior to screening.

Study Drug and Doses

rAvPAL-PEG for subcutaneous injection, supplied as a sterile aqueous (colorless to pale yellow) solution at a concentration of 10 mg/mL, was administered by study personnel on Day 1 at a dose of 0.001, 0.003, 0.01, 0.03, and 0.1 mg/kg to subjects with PKU. Since this was the first study in humans, doses were selected based on animal studies ranging from one very low to test drug safety and not expected to produce a decrease in phenylalanine levels to one capable of therapeutic efficacy.

Safety and Tolerability Assessments

Safety was assessed by examining the incidence of all adverse events (AEs) reported during the study period and clinically significant changes in vital signs and clinical laboratory results, including development of rAvPAL-PEG antibodies.

All subjects had safety monitoring including vital signs (temperature, heart rate, respiratory rate, blood pressure) with pulse oximetry on a daily basis before and during the first 5 days after the injection, physical examination on screening and Days 1 and 15, and routine clinical laboratory tests (CBC, chemistries, and urinalysis) on screening and Days 1, 2, 8 and 15. Electrocardiogram and other laboratory tests (urine pregnancy test, urine drug screen, and plasma ammonia) were also performed during the study. All subjects had a safety follow-up phone contact two weeks following the completion of the study treatment. Immunoglobulin G (IgG) and M (IgM) specific for rAvPAL and PEG and neutralizing antibodies (NAbs) specific for rAvPAL-PEG were measured on Days 1, 15, 29 $\pm$ 2 and 36 $\pm$ 2.

Blood Phe concentrations were obtained 2.5 hours after a meal on screening, pre-dose and 8 hours post-dose on day 1 and daily thereafter. Blood Phe concentrations were assessed at local laboratories for each site using ion-exchange chromatography or liquid chromatography/mass spectrometry/mass spectrometry. The different laboratories reported similar values of Phe on proficiency samples tested before initiation of the study, as is usually the case for Phe monitoring¹⁶. All samples for each individual patient were analyzed by the same laboratory.

Statistical Analysis

Safety was assessed by examining the incidence of all AEs reported during the study period and clinically significant changes in vital signs or clinical laboratory results, including the development of rAvPAL-PEG antibodies. AEs were coded in accordance with the *Medical Dictionary for Regulatory Activities* (MedDRA), version 10.0 and reported descriptively.

Formal power analysis was not performed. The change in blood Phe concentration from baseline (predose, Day 1) over time was evaluated by comparing the average Phe concentrations at different timepoints and different doses to the concentration at baseline using a paired t-test. Efficacy was defined as a significant reduction from baseline of phenylalanine levels. The linear correlation between Phe and drug concentrations in the blood was established using analysis of variance, using $p < 0.05$ to define a significant relationship.

Pharmacokinetics Analysis

Pharmacokinetic (PK) analyses of plasma rAvPAL-PEG concentration-time data were performed for all subjects using non-compartmental methods to obtain estimates of PK parameters. Peak plasma concentrations ($C_{\max}$) and time to peak concentration ($T_{\max}$) were obtained directly from the data without interpolation; λ_z , the apparent terminal elimination rate constant, was determined by log-linear regression of the terminal plasma concentrations; the area under the plasma concentration-time curve from time zero to the time of last measurable concentration (AUC_{0-t}) was calculated by the linear trapezoidal method; and the area under the plasma concentration-time curve from time zero to infinity ($AUC_{0-\infty}$) was calculated where $AUC_{0-\infty} = AUC_{0-t} + C_t / \lambda_z$ and C_t is the last measurable concentration.

The plasma concentration-time data were analyzed using the software WinNonlin Professional (Pharsight Corporation, Version 5.2) to perform non-compartmental PK modeling. Calculations such as AUC_{0-t} ratios and descriptive statistics were performed using Microsoft Excel (Version 11.0). Concentrations of the drug < 25 ng/mL were set at zero for PK and statistical analysis and plotting.

Role of the funding source

The sponsor designed the study, provided central data collection, and performed initial data analysis and interpretation of pharmacokinetic results. The medical writer created a first draft of the manuscript. The corresponding author had full access to all the data in the study, analyzed data, created new graphs, provided interpretation and had final responsibility for the decision to submit for publication. All co-authors contributed patients and data to the study, helped in data interpretation, and reviewed the final manuscript.

Results

Safety

The 25 subjects with PKU had increased Phe concentrations (baseline 1310 ± 405 $\mu\text{mol/L}$) and mildly increased average body mass index (26.4 ± 4.6), with normal values for other measured parameters (Table 1). Twenty of the 25 subjects (80%) who received the single dose of the study drug reported at least one AE during the study (Table 2). A possible or probable relationship to treatment with rAvPAL-PEG was indicated for the majority of subjects (16 subjects [64%]) (Table 2). A total of 11 subjects (44%) reported at least one AE during the study that was categorized as an injection-site reaction following administration of rAvPAL-PEG, with 5 (20%) classified as injection-site bruising and 4 (16%) as an injection site reaction (Table 3). Other reactions included erythema, pain, rash, swelling, and urticaria which occurred in the majority of subjects (4/5) receiving the highest dose. All of these injection-site reactions resolved spontaneously without specific treatment. Additional frequently reported AEs (incidence $> 10\%$ or reported by ≥ 3 subjects) were nervous system disorders consisting of dizziness and headache (9 reports [36%]); infections and infestations (7 reports [28%]) and nasopharyngitis (3 reports [12%]); musculoskeletal and connective tissue disorders (7 reports [28%]); gastrointestinal (GI) disorders (6 reports [24%]); injury, poisoning, and procedure complications and skin and SC tissue disorders (4 reports per body

system [16%]); and respiratory, thoracic, and mediastinal disorders and immune system disorders (3 reports per body system [12%]). Musculoskeletal and connective tissue disorders (arthralgia, musculoskeletal pain, extremity pain, and muscle spasms) attributed to treatment with rAvPAL-PEG were reported for more subjects who received a dose of 0.1 mg/kg (three subjects [60%]) than subjects who received 0.001 mg/kg (one subject [20%]) or 0.003-0.03 mg/kg (0 subjects).

Three subjects reported hypersensitivity AE following administration of rAvPAL-PEG. One subject who received a dose of 0.003 mg/kg reported a non-serious hypersensitivity AE that was mild in severity and possibly related to treatment with rAvPAL-PEG. The two other subjects had a reaction after receiving IM injections of medroxyprogesterone (Depo-Provera®), a PEGylated contraceptive after the study drug. These two subjects reported serious AEs (SAEs) that were assessed as moderate in severity and related to treatment with rAvPAL-PEG (Table 2). Both SAEs were categorized as immune system disorders of anaphylactic reaction (urticaria for one subject who received 0.01 mg/kg) and hypersensitivity (an allergic reaction for one subject who received 0.001 mg/kg) (Table 3). One of the subjects also received the human papillomavirus vaccine (Gardasil®) 15 days following the administration of rAvPAL-PEG. One subject who received 0.03 mg/kg reported two AEs that were severe (myalgia and muscle strain), neither of which was assessed as related to treatment with the study drug and these resolved spontaneously. For the 19 other subjects who reported an AE during the study, the events were mild or moderate in severity.

No subject reported a pregnancy, died or discontinued the study early (Table 2). Laboratory test results, including plasma ammonia levels, CH50, urinalysis, and serum chemistry, were within the normal range, with no trends or clinically significant changes from baseline over time for all subjects. In the subjects in whom they were measured, plasma tyrosine levels were below normal before the injection of the drug and had no significant changes after the injection. There were no clinically significant changes in vital sign measurements during the study. None of the 25 subjects had a clinically significant ECG abnormality and no differences were observed across the five dosages.

Immunogenicity

The immunologic response to rAvPAL-PEG can be directed toward PAL and/or PEG. Both types of antibodies were measured in the treated population (Tables 4 and 5; Figure 1). Anti-PAL antibodies were present in about 20% of subjects at baseline (4% with IgM and 16% with IgG). Fortythree days after the injection, 44% of subjects had anti-PAL antibodies (8% with IgM and 36% with IgG) (Table 4). Neutralizing anti-PAL antibodies were not detected prior or after the injection. Anti-PEG antibodies were present in 16% of subjects at baseline (4% IgM, 12% IgG) and in 100% of subjects 43 days after the injection with 72% of subjects developing IgM (18/25 subjects) and 100% developing IgG. There was no clear relationship between appearance of antibodies and dose of rAvPAL-PEG, AEs, or injection-site reactions after a single dose of rAvPAL-PEG.

Effect of rAvPAL-PEG on Phenylalanine Concentrations

Figure 2 reports plasma Phe concentrations in subjects with PKU as a function of time with different doses of rAvPAL-PEG. No clinically significant changes in Phe concentrations were observed in the first four dosing groups (rAvPAL-PEG dose: 0.001 to 0.03 mg/kg). Significantly reduced Phe concentrations as compared to baseline were observed in subjects receiving 0.1 mg/kg rAvPAL-PEG from Day 4 to Day 7 following drug administration ($p < 0.05$).

At a dose of 0.1 mg/kg, rAvPAL-PEG reduced mean plasma Phe concentrations from $1113 \pm 323 \mu\text{mol/L}$ at baseline to $575 \pm 390 \mu\text{mol/L}$ on Day 6 ($-537 \pm 134 \mu\text{mol/L}$ compared to baseline) after the injection with reduced Phe levels being maintained for 5-7 days following the single-dose administration (Figure 2). After Day 11, mean blood Phe concentrations steadily increased and returned to baseline concentrations by the end of the study (Day 43, $1185 \pm 213 \mu\text{mol/L}$).

Pharmacokinetics Results

Subjects who received rAvPAL-PEG at doses of 0.01, 0.03, and 0.1 mg/kg had measurable rAvPAL-PEG levels in plasma, whereas rAvPAL-PEG levels were below the detection limit at the lower doses (0.001 and 0.003 mg/kg). The calculated PK parameters with a dose of 0.01, 0.03 and 0.1 mg/kg were a mean T_{max} of between 89–106 hours, a $T_{1/2}$ of 46–120 hours, and an AUC_{0-t} and C_{max} that were proportional to the dose, with the highest values in subjects receiving the highest dose. Exposure was slightly greater with increased dose. The drug concentration dropped below the lower limit of quantitation starting from 200 hours (in subjects receiving a dose of 0.01 mg/kg) and up to 336 hours (0.1 mg/kg) after the injection. The maximal decrease in Phe concentrations for subjects receiving 0.1 mg/kg occurred about one day after the highest plasma concentration of rAvPAL-PEG (T_{max}) was reached at 100.8 hours (Figure 3A). For subjects who received 0.1 mg/kg, there was a statistically significant inverse correlation between the plasma concentrations of rAvPAL-PEG and Phe (Figure 3B). This inverse correlation was observed in each subject and when all data were considered together.

Discussion

Treatment with rAvPAL-PEG administered as a single subcutaneous dose (0.1 mg/kg) reduced blood Phe concentrations from baseline and maintained the reduction for up to five days post-injection in subjects with PKU, with no significant reduction in blood Phe concentrations observed at the lower doses (0.001, 0.003, 0.01, and 0.03 mg/kg) (Figure 2). The maximal reduction of Phe concentrations ($51.6 \pm 36.4\%$ of baseline) occurred 6 days after the injection. rAvPAL-PEG reached the maximum concentration at 89–106 hours following administration of 0.1 mg/kg rAvPAL-PEG, with a half-life of 46–120 hours and an AUC and C_{max} that was proportional to the dose. There was a statistically significant relationship between the plasma concentrations of rAvPAL-PEG and reduction in Phe levels in subjects with PKU who received 0.1 mg/kg of the study drug (Figure 3B), demonstrating a dose-dependent effect.

Doses of rAvPAL-PEG at 0.001, 0.003, 0.01, 0.03, or 0.1 mg/kg were well tolerated in all 25 subjects studied. No subject died, suffered substantial permanent morbidity or discontinued the study prematurely following rAvPAL-PEG injection. Adverse events and injection-site reactions were more frequent at the highest dose (0.1 mg/kg) suggesting a relationship with the dose of drug administered, although the precise relationship between drug dose and these reported AEs remains unknown due to the small sample size. Two SAEs were reported during this study; neither was severe and both resolved with treatment (Table 2). These SAEs were reported for subjects receiving the lower doses (0.001 mg/kg and 0.01 mg/kg) who had previous and concomitant administration of a PEG-containing drug (medroxyprogesterone acetate injection [Depo-Provera[®]]). Both subjects had negligible anti-PEG IgG antibody titer results at baseline that increased after administration of the study drug, potentially contributing to the reactions following the single-dose administration of rAvPAL-PEG. Following these reactions, enrollment in this Phase 1 study was restricted to subjects who were not taking any PEG-containing injectable medications.

Anti-PEG antibodies were present in a significant proportion of subjects at baseline, likely reflecting the widespread presence of PEG in the environment (Tables 4, and 5, and Figure 1). The two subjects with the highest anti-PEG IgG titers (36,450 DF) during the study developed a reaction with medroxyprogesterone acetate or a generalized skin reaction, suggesting that the administration of rAvPAL-PEG at any dose may have induced antibody titers and may therefore have initiated the observed immune responses. Apart these two cases, no clear-cut relationship was observed between adverse events and antibody titers.

The limitations of this study are in the low number of subjects studied, the possible effects of confounding variables (such as changes in the diet), and the lack of knowledge on the effects of repeated drug administration.

The results from this first-in-human study establish that rAvPAL-PEG in a single dose of up to 0.1 mg/kg is relatively safe and well tolerated and can significantly reduce Phe concentrations in subjects with PKU. Clinical availability of rAvPAL-PEG could greatly improve maintenance of Phe levels within the recommended range in subjects with phenylketonuria and improve their neuropsychological outcome. Phase II studies are necessary to assess the effect of repeat dosing of rAvPAL-PEG in subjects with PKU given the immunologic data showing appearance of anti-PEG antibodies.

Research in Context

Systematic review

Phenylalanine ammonia lyase is a non-mammalian enzyme that degrades phenylalanine and could reduce elevated phenylalanine levels in patients with phenylketonuria. Preclinical models strongly suggest that injection of this drug can reduce phenylalanine levels in a mouse model of phenylketonuria¹⁵. We did a first-in-human trial to investigate the safety, tolerability, and efficacy of single-dose rAvPAL-PEG (PEGylated recombinant phenylalanine ammonia lyase) in patients with phenylketonuria.

Interpretation

rAvPAL-PEG was relatively safe and well tolerated and was effective in significantly reducing phenylalanine concentrations in subjects with phenylketonuria at a single dose of 0.1 mg/kg. Additional studies are necessary to assess the effect of repeat dosing of rAvPAL-PEG in subjects with phenylketonuria given the appearance of anti-PEG antibodies after a single injection.

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Figure 1. Appearance of anti-PEG (panel A) and anti-PAL (panel B) antibodies in subjects with phenylketonuria after a single injection of rAvPAL-PEG in subjects with phenylketonuria
 All subjects developed antibodies to PEG, whereas less than 50% had a response to PAL.
 No differences in the rate of antibody formation were seen across different doses. Anti-PEG: percent of subjects who developed either IgG or IgM against PEG; Anti-PAL: percent of subjects who developed either IgG or IgM against PAL.

Figure 2. Effect of increasing doses (0.001–0.1 mg/kg) of rAvPAL-PEG on plasma phenylalanine concentrations in subjects with phenylketonuria

Subjects received a single dose of the drug on Day 1. Points are averages $\pm$ standard deviation (SD) of five determinations. * $p < 0.05$ versus baseline using paired t-test.

Figure 3. Panel A: Concentrations of plasma phenylalanine (filled circle) and rAvPAL-PEG (open squares) in subjects with phenylketonuria after one dose (time zero) of 0.1 mg/kg of rAvPAL-PEG

Points are averages with the SD indicated in one direction. **Panel B: Correlation between plasma concentrations of rAvPAL-PEG and phenylalanine.** Note the significant inverse correlation between plasma rAvPAL-PEG and phenylalanine concentrations in subjects with phenylketonuria who received a single dose of 0.1 mg/kg of rAvPAL-PEG. The thick line represents the regression using all points with the parameters indicated. The lines are regression to individual subjects. These were all highly significant ($p<0.01$) except in Subject 5 where the regression was statistically significant ($p<0.05$).

Table 1

Baseline characteristics of subjects with phenylketonuria who received rAvPAL-PEG.

Variable	Mean $\pm$ SD
Age (years)	28.4 $\pm$ 7.4
Pre-dose blood phenylalanine (μ mol/L)	1310 $\pm$ 405
Weight (kg)	75.3 $\pm$ 14.9
Height (cm)	168.9 $\pm$ 8.8
Body mass index (BMI)	26.4 $\pm$ 4.6
Systolic blood pressure (SBP)	115.5 $\pm$ 11.8
Diastolic blood pressure (DBP)	68.2 $\pm$ 6.7
Gender (% male)	15 (60%)
Ethnicity (% white)	24 (96%)

Table 2

Adverse events possibly or probably related to administration of rAvPAL-PEG.

Type of AE	rAvPAL-PEG Dose Group						Combined(n=25)
	Group 1 0.001 mg/ kg(n=5)	Group 2 0.003 mg/ kg(n=5)	Group 3 0.01 mg/ kg(n=5)	Group 4 0.03 mg/ kg(n=5)	Group 5 0.1 mg/ kg(n=5)		
Any AEs ^a	4 (80%)	3 (60%)	4 (80%)	4 (80%)	5 (100%)		20 (80%)
Any drug-related AEs	4 (80%)	3 (60%)	3 (60%)	2 (40%)	4 (80%)		16 (64%)
Any SAEs ^b	1 (20%)	0 (0%)	1 (20%)	0 (0%)	0 (0%)		2 (8%)
Any drug-related SAEs	1 (20%)	0 (0%)	1 (20%)	0 (0%)	0 (0%)		2 (8%)
Any AEs causing study discontinuation	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)		0 (0%)
Death	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)		0 (0%)

^a AEs: adverse events

^b SAEs: serious adverse events

Table 3

Adverse events that were reported in more than 10% of the population of subjects with phenylketonuria who received a single dose of rAAV-PAL-PEG.

	Group 1 0-001mg/kg(n=5)	Group 2 0-003mg/kg(n=5)	Group 3 0-01mg/kg(n=5)	Group 4 0-03mg/kg(n=5)	Group 5 0-1mg/kg(n=5)	Combined(n=25)
Subjects with at least one reported AE*	4 (80%)	3 (60%)	4 (80%)	4 (80%)	5 (100%)	20 (80%)
General disorders and administration site conditions	1 (20%)	2 (40%)	2 (40%)	3 (60%)	4 (80%)	12 (48%)
Injection-site bruising	1 (20%)	1 (20%)	1 (20%)	0	2 (40%)	5 (20%)
Injection-site reaction	0	1 (20%)	0	1 (20%)	2 (40%)	4 (16%)
Nervous system disorders	3 (60%)	2 (40%)	1 (20%)	3 (60%)	0	9 (36%)
Dizziness	3 (60%)	0	1 (20%)	1 (20%)	0	5 (20%)
Headache	0	2 (40%)	0	2 (40%)	0	4 (16%)
Infections and infestation	2 (40%)	1 (20%)	2 (40%)	1 (20%)	1 (20%)	7 (28%)
Nasopharyngitis	2 (40%)	0	0	1 (20%)	0	3 (12%)
Musculoskeletal and connective tissue disorder	2 (40%)	0	1 (20%)	1 (20%)	3 (60%)	7 (28%)
Arthralgia	1 (20%)	0	0	0	2 (40%)	3 (12%)
Pain in extremity	1 (20%)	0	0	0	2 (40%)	3 (12%)
Gastrointestinal disorders	1 (20%)	0	2 (40%)	3 (60%)	0	6 (24%)
Injury, poisoning, and procedural complications	1 (20%)	0	2 (40%)	1 (20%)	0	4 (16%)
Skin and SC [‡] tissue disorders	0	0	0	1 (20%)	3 (60%)	4 (16%)
Immune system disorders	1 (20%)	1 (20%)	1 (20%)	0	0	3 (12%)
Hypersensitivity	1 (20%)	1 (20%)	1 (20%)	0	0	3 (12%)

Subjects who experienced more than one AE within a given MedDRA[†] preferred term were counted once within that MedDRA preferred term. All subjects who enrolled in the study were included in the safety population.

* AE: adverse events

[†] MedDRA: Medical Dictionary for Regulatory Activities

[‡] SC: subcutaneous

Table 4
Anti-PAL antibodies: Subjects with phenylketonuria with a positive antibody response to rAvPAL-PEG.*

Dose (mg/kg)	0-001 (n=5)	0-003 (n=5)	0-01 (n=5)	0-03 (n=5)	0-1 (n=5)	Combined (n=25)
Any PAL antibody						
Baseline	2/5 (40%)	1/5 (20%)	2/5 (40%)	0/5	0/5	5/25 (20%)
Day 15	1/5 (20%)	1/5 (20%)	2/5 (40%)	2/5 (40%)	4/5 (80%)	10/25 (40%)
Day 29	1/5 (20%)	1/5 (20%)	1/3 (33%)	0/5	4/5 (80%)	7/23 (30%)
Day 43	2/5 (40%)	1/5 (20%)	2/5 (40%)	2/5 (40%)	4/5 (80%)	11/25 (44%)
Anti-PAL IgM [†]						
Baseline	1/5 (20%)	0/5	0/5	0/5	0/5	1/25 (4%)
Day 15	0/5	0/5	0/5	1/5 (20%)	1/5 (20%)	2/25 (8%)
Day 29	1/5 (20%)	0/5	0/3	0/5	0/5	1/23 (4%)
Day 43	1/5 (20%)	0/5	0/5	1/5 (20%)	0/5	2/25 (8%)
Anti-PAL IgG [‡]						
Baseline	1/5 (20%)	1/5 (20%)	2/5 (40%)	0/5	0/5	4/25 (16%)
Day 15	1/5 (20%)	1/5 (20%)	2/5 (40%)	2/5 (40%)	4/5 (80%)	10/25 (40%)
Day 29	0/5	1/5 (20%)	1/3 (33%)	0/5	4/5 (80%)	6/23 (26%)
Day 43	1/5 (20%)	1/5 (20%)	2/5 (40%)	1/5 (20%)	4/5 (80%)	9/25 (36%)

* Percentages are based on the number of subjects within a group with available antibody data at the indicated drug dose.

[†] IgM: immunoglobulin M

[‡] IgG: immunoglobulin G

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