CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

761079Orig1s000

MULTI-DISCIPLINE REVIEW

Summary Review
Office Director
Cross Discipline Team Leader Review
Clinical Review
Non-Clinical Review
Statistical Review
Clinical Pharmacology Review

BLA Multi-Disciplinary Review and Evaluation
BLA 761079 PALYNZIQ (pegvaliase-pqpz)

BLA Multi-Disciplinary Review and Evaluation

Application Type	Biologics License Application (BLA)
Application Number(s)	761079
Priority or Standard	Priority with 3 Month Extension due to Major Amendment
Submit Date(s)	June 30, 2017 (eCTD SN0001)
Received Date(s)	June 30, 2017 (eCTD SN0001)
PDUFA Goal Date	May 28, 2018
Action Goal Date	May 25, 2018
Action Date	May 24, 2018
Division/Office	Division of Gastroenterology and Inborn Errors Products (DGIEP)/ Office of Drug Evaluation III (ODE III)
Review Completion Date	May 24, 2018
Established Name	pegvaliase-pqpz
(Proposed) Trade Name	PALYNZIQ
Pharmacologic Class	enzyme substitution therapy (phenylalanine-metabolizing enzyme)
Code name	BMN 165
Applicant	BioMarin Pharmaceutical, Inc.
Formulation(s)	Clear sterile solution
Dosing Regimen	Induction, Titration, Maintenance Regimen (start at 2.5 mg once weekly and titrate up to 20 mg once daily or 40 mg once daily)
Applicant Proposed Indication(s)/Population(s)	To reduce blood phenylalanine concentrations in adult patients with phenylketonuria who have uncontrolled blood phenylalanine concentrations > 600 micromol/L on existing management
Recommendation on Regulatory Action	Approval
Recommended Indication(s)/Population(s) (if applicable)	To reduce blood phenylalanine concentrations in adult patients with phenylketonuria (PKU) who have uncontrolled blood phenylalanine concentrations greater than 600 micromol/L on existing management

BLA Multi-Disciplinary Review and Evaluation
BLA 761079 PALYNZIQ (pegvaliase-pqpz)

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OND: Office of New Drugs
ODE: Office of Drug Evaluation
DGIEP: Division of Gastroenterology and Inborn Errors Products
OTS: Office of Translational Sciences
OCP: Office of Clinical Pharmacology
DCP: Division of Clinical Pharmacology
DPM: Division of Pharmacometrics
OB: Office of Biostatistics
DB: Division of Biometrics
OSE: Office of Surveillance and Epidemiology
OMEPRM: Office of Medication Error Prevention and Risk Management
DRISK: Division of Risk Management

Additional Review Disciplines

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OND/ODEIII/DGIEP	Fang Cai	DARRTS (Reference ID: 4230623)
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OPQ/OPF/DMA	Patricia Hughes / Maria (Reyes) Candauchacon / Lindsey Brown / Maxwell Van Tassell	CDER Informatics Platform/Panorama
OPQ/OPF/DIA	Thuy Thanh Nuguyen / Zhihao Peter Qiu / Ephrem Hunde	CDER Informatics Platform/Panorama
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OND/ODEIV/DPMH	Lynne Yao / Miriam Dinatale / Catherine Roca / Jacquline Yancy	DARRTS (Reference ID: 4226692)
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DDS: Deputy Director for Safety
ADL: Associate Director for Labeling
OPQ: Office of Pharmaceutical Quality
OBP: Office of Biotechnology Products
OPF: Office of Process and Facilities
DMA: Division of Microbiology Assessment
DIA: Division of Inspectional Assessment
DBRR: Divisions of Biotechnology Review and Research
OPRO: Office of Program and Regulatory Operations
DRBPM: Division of Regulatory and Business Process Management
DPARP: Division of Pulmonary, Allergy, and Rheumatology Products
DCRP: Division of Cardiovascular and Renal Products
DPMH: Division of Pediatric and Maternal Health
DMEPA: Division of Medication Error Prevention and Analysis
OPE: Office of Pharmacovigilance and Epidemiology
DEPI: Division of Epidemiology
DPV: Division of Pharmacovigilance
PMS: Project Management Staff
CBER: Center for Biologics Evaluation and Research
CDRH: Center for Devices and Radiological Health
OMP: Office of Medical Policy
OPDP: Office of Prescription Drug Promotion
OMPI: Office of Medical Policy Initiatives
DMPP: Division of Medical Policy Programs
OC: Office of Compliance
OSI: Office of Scientific Investigations
OSIS: Office of Study Integrity and Surveillance

Glossary

ADME	Absorption, Distribution, Metabolism, Excretion
AE	Adverse Event
BLA	Biologics License Application
BPCA	Best Pharmaceuticals for Children Act
BRF	Benefit Risk Framework
CBER	Center for Biologics Evaluation and Research
CDER	Center for Drug Evaluation and Research
CDRH	Center for Devices and Radiological Health
CDTL	Cross-Discipline Team Leader
CFR	Code of Federal Regulations
CMC	Chemistry, Manufacturing, and Controls
CNS	Central Nervous System
CRO	Contract Research Organization
CSR	Clinical Study Report
DARRTS	Document Archiving, Reporting, and Regulatory Tracking System
DMC	Data Monitoring Committee
DPARP	Division of Pulmonary, Allergy, and Rheumatology Products
ECG	Electrocardiogram
eCTD	Electronic Common Technical Document
ETASU	Elements to Assure Safe Use
FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act of 2007
FDASIA	Food and Drug Administration Safety and Innovation Act
GCP	Good Clinical Practice
ICH	International Conference on Harmonization
IEM	Inborn Error of Metabolism
IND	Investigational New Drug
ISE	Integrated Summary of Effectiveness
ISS	Integrated Summary of Safety
ITT	Intent to Treat
MedDRA	Medical Dictionary for Regulatory Activities
mITT	Modified Intent to Treat
NCI-CTCAE	National Cancer Institute-Common Terminology Criteria for Adverse Events
NME	New Molecular Entity
OCS	Office of Computational Science
OPQ	Office of Pharmaceutical Quality
OSE	Office of Surveillance and Epidemiology

OSI	Office of Scientific Investigation
PAH	Phenylalanine Hydroxylase
Phe	Phenylalanine
PD	Pharmacodynamic
PI	Prescribing Information
PK	Pharmacokinetic
PKU	Phenylketonuria
PMC	Postmarketing Commitment
PMR	Postmarketing Requirement
PP	Per Protocol
PPI	Patient Package Insert
PREA	Pediatric Research Equity Act
PRO	Patient Reported Outcome
rAvPAL	recombinant *Anabaena variabilis* phenylalanine ammonia lyase
REMS	Risk Evaluation and Mitigation Strategy
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SC	Subcutaneously
SOC	Standard of Care
TEAE	Treatment Emergent Adverse Event

1 Executive Summary

1.1 Product Introduction

PALYNZIQ (pegvaliase-pqpz), an original biologic, is a phenylalanine-metabolizing enzyme produced through the PEGylation of recombinant phenylalanine ammonia lyase (rAvPAL), which is derived from the cyanobacterium *Anabaena variabilis*. Pegvaliase substitutes for the deficient phenylalanine hydroxylase (PAH) enzyme in patients with Phenylketonuria (PKU) by providing an alternate pathway for phenylalanine (Phe) breakdown via the enzymatic conversion of Phe to trans-cinnamic acid (t-CA) and ammonia, both excreted in the urine.

Pegvaliase is administered as a subcutaneous (SC) injection through a single-dose, prefilled syringe. The proposed dosing regimen includes sequentially an induction, titration, and maintenance (I/T/M) phase during which the dose is slowly increased from a starting dose of 2.5 mg once weekly to a target maintenance dose of 20 mg once daily over a period of at least 9 weeks. The Applicant proposes that a patient should stay on 20 mg once daily for at least 24 weeks, after which the dose may be increased to 40 mg once daily if an adequate therapeutic response (at least a 20% reduction in blood Phe concentration from pre-treatment baseline or a blood Phe concentration ≤ 600 micromol/L) is not reached on the 20 mg daily dose. If a patient does not achieve at least a 20% reduction in blood Phe concentration from pre-treatment baseline or a blood Phe concentration ≤ 600 micromol/L after an additional 16 weeks of continuous treatment with 40 mg once daily, then pegvaliase treatment should be discontinued. Dose titration and dose escalation should be directed by blood Phe concentrations and patient tolerability.

1.2 Conclusions on the Substantial Evidence of Effectiveness

The Applicant has provided substantial evidence of effectiveness, as required by 21 CFR 314.126(a)(b), to support approval of Palynziq (pegvaliase-pqpz) for the treatment of adult patients with PKU who have uncontrolled blood phenylalanine (Phe) concentrations greater than 600 micromol/L on existing management. The Applicant has demonstrated that Palynziq given through an induction, titration, and maintenance dosage regimen with a target dose of either 20 mg SC once daily or, at a maximum, 40 mg SC once daily (depending on individual therapeutic response and tolerability) is effective in reducing blood Phe concentrations in adult patients with PKU.

1.3 Benefit-Risk Assessment

Benefit-Risk Summary and Assessment

PALYNZIQ (pegvaliase-pqpz) is a phenylalanine-metabolizing enzyme intended to be used as an enzyme substitution therapy. The proposed indication is to reduce blood phenylalanine (Phe) concentrations in adult patients with PKU who have uncontrolled blood Phe concentrations (blood Phe > 600 micromol/L) on existing management. PALYNZIQ treatment resulted in statistically and clinically significant reductions in blood Phe concentrations in adults with PKU treated in the phase 3 trials. After thorough review of this application, we recommend approval for PALYNZIQ at target maintenance doses of 20 mg subcutaneously (SC) once daily and 40 mg SC once daily (maximum dose) for the Applicant's proposed indication. The target maintenance dosage should be based on individual patient therapeutic response and tolerability.

Phenylketonuria (PKU) is a serious, rare, inherited disease which manifests with chronic hyperphenylalaninemia leading to chronic neurologic and psychiatric disease when untreated or undertreated. Metabolic control through reduction in blood Phe concentrations throughout the patient's lifetime is the overall therapeutic goal in PKU management. Available therapeutic options for PKU management include: 1) the PKU diet, which consists of lifetime strict restriction of dietary protein and Phe intake, and 2) Kuvan (sapropterin dihydrochloride), the PAH enzyme cofactor, which is indicated only for those patients with PKU who are "Kuvan responsive" (approximately 30% of all PKU patients). Patients with the classical (severe) form of PKU who exhibit the highest blood Phe concentrations, when untreated, typically need the strictest protein and Phe intake restriction to maintain metabolic control, and are the least likely to respond to Kuvan (about 10% of classical PKU patients are Kuvan responders).Control of blood Phe concentrations within the generally recommended range (below 600 micromol/L, ideally within 120-360 micromol/L) is especially challenging for adults with PKU owing to the difficulty of adhering to the strict lifelong dietary restrictions required to maintain optimal metabolic control. Poor adherence to the dietary management in PKU results in adverse clinical outcomes from untreated or undertreated disease which include neurologic and psychiatric disease (executive dysfunction, attention and memory impairment, and chronic psychiatric disease). Thus, an unmet medical need exists for adults with PKU, particularly for patients with the classical form of the disease, as these patients are unable to adhere to the very strict restrictions of the PKU diet, and Kuvan is not a viable therapeutic option for them.

The efficacy of PALYNZIQ, reflected in reduction of blood Phe concentrations from pre-treatment baseline, was assessed in two Phase 3 clinical

trials. In the first trial, Trial 165-301, a randomized (to two target maintenance doses), open-label clinical trial, treatment-naïve PKU patients were treated through an induction/titration/maintenance dosage regimen starting with 2.5 mg once weekly dose and titrating up over several weeks to a target maintenance dose of either 20 mg once daily or 40 mg once daily (depending on the randomization arm; no placebo arm included). This trial enrolled adults with PKU who had baseline blood Phe concentrations > 600 micromol/L on existing dietary management. The median pre-treatment blood Phe concentration in the trial was 1,221 micromol/L (range 285-2,330 micromol/L), indicating poor metabolic control, and 84% were maintained on an unrestricted diet prior to treatment initiation. For patients randomized to the target dose of 20 mg once daily, the time to achieve a therapeutic response (blood Phe reduction ≥ 20% from pretreatment baseline or blood Phe concentration ≤600 micromol/L) ranged between 4-24 weeks on the 20 mg once daily dose. Notably, of the patients who started treatment while on dietary protein restriction (which constituted the minority), most liberalized their diet (increased their protein intake) during PALYNZIQ treatment in Trial 165-301 while maintaining a therapeutic response (blood Phe concentration below their pre-treatment baseline).

PALYNZIQ was compared to placebo in a second Phase 3 trial, Trial 165-302, which had a complex design consisting of 4 parts. In "part 2," which had a randomized withdrawal design and served to demonstrate primary efficacy, used an enrichment strategy and selected treatment responders (those patients, primarily treated in Trial 165-301, who had ≥20% reduction in blood Phe concentration from pre-treatment baseline). Patients were randomized to either continue their target maintenance dose of 20 mg once daily or 40 mg once daily (to which they were randomized in Trial 165-301) or to switch to a matching placebo ("placebo 20 mg daily" or "placebo 40 mg daily") for 8 weeks. Patients who were randomized to continue their previous dose preserved their treatment response (blood Phe concentration) while those who switched to matching placebo returned to their pre-treatment Phe concentrations after 8 weeks of treatment withdrawal. The differences between each active treatment and each matching placebo groups were statistically significant (p-value <0.0001).

Overall, therapeutic response varied among patients in the phase 3 trials and no single patient characteristic was found to be predictive of therapeutic response. However, most patients achieved at least a 20% reduction or a blood Phe concentration below 600 micromol/L within the first 4 weeks of treatment with 20 mg once daily in Trial 165-301. There was a delay in therapeutic response in some patients beyond this initial 4 weeks with Phe improvements seen after 24 weeks of treatment with 20 mg once daily (during Trial 165-302 "part 4," which was an open-label extension). Patients who were initially randomized to a target dose of 20 mg once daily in Trial 165-301 and whose blood Phe concentration remained above 600 micromol/L were further escalated to 40 mg once daily in" part 4" of Trial 165-302. Of those who dose-escalated from 20 mg once daily to 40 mg once daily, half achieved blood Phe reduction to below 600 micromol/L after an additional 16 weeks of treatment with 40 mg once daily.

As previously stated, the therapeutic goal in PKU management is metabolic control via blood Phe reduction (at a minimum to < 600 micromol/L) and this goal cannot be achieved in the absence of strict dietary protein restriction. As such and given that the observed blood Phe reductions in the pegvaliase Phase 3 trials were achieved while largely on no dietary protein restriction, this therapeutic effect constitutes a clinically significant achievement. In fact, the trial population included patients with mean baseline blood Phe concentration of >1,200 micromol/L, indicating poor metabolic control and a range seen mostly in untreated classical PKU, a population with the highest need for new therapeutic options. In addition, even though the magnitude of blood Phe reduction varied among patients in the context of blunting of the therapeutic effect due to the product's immunogenicity, the majority of patients achieved a significant therapeutic response (≥20% reduction in blood Phe concentration or a blood Phe concentration below 600 micromol/L) within a reasonable amount of time (up to 24 weeks on 20 mg once daily and up to an additional 16 weeks on 40 mg once daily for a total of up to 40 weeks of treatment). In fact, some patients achieved further blood Phe reduction with Phe concentrations within the generally recommended and more strict range of 120-360 micromol/L, as in accordance with the American College of Medical Genetics and Genomics PKU management practice guidelines. A few patients even experienced an exaggerated therapeutic response with evidence of hypophenylalaninemia (blood Phe concentration below 30 micromol/L), a finding rarely seen in PKU adults while on dietary restriction alone; these events of hypophenylalaninemia were managed by appropriate dietary modification and/or dose reduction and appeared to recover without clinical sequelae.

The safety of pegvaliase was assessed throughout short-term and long-term treatment in the phase 3 trials (mean exposure of 2 years with a few patients treated for over 4 years). Patients experienced adverse events predominantly associated with hypersensitivity to the product, which is not unexpected given that rAvPAL is a foreign protein. There were no product-related deaths. Serious adverse events (SAEs), probably or possibly related to the product, included: 37 anaphylaxis events, 26 hypersensitivity events, 6 events of elevated CPK, 6 events of chest pain, 2 events of generalized skin reaction lasting at least 14 days, 1 event of arthralgia, and 1 event of injection-site reaction. Anaphylaxis occurred in 26 patients (9%) and about half were treated with injectable epinephrine. All patients who experienced anaphylaxis fully recovered from the event without sequelae. Over 90% of patients experienced at least one hypersensitivity AE, most of which were reported as mild to moderate in severity and were managed with antihistamines, NSAIDs, and/or corticosteroids. The most frequently reported TEAEs (adjusted for the variable time of exposure) included: injection-site reactions, generalized skin reactions lasting at least 14 days, arthralgia, hypersensitivity reactions, and headache.

All patients in the pegvaliase phase 3 trials developed a high and sustained immune response to the product manifesting as IgM and IgG antibodies to the PAL protein and to the PEG component of the product. Immune-mediated laboratory abnormalities reported in over 50% of patients included low complement C3 and C4 concentrations and high C-Reactive Protein (CRP) on at least one measurement. Efficacy

appeared to be affected by antibody-mediated enhanced drug clearance and/or inhibition of the enzyme activity. However, no specific antibody response was predictive of therapeutic response in the trials. While no single immunologic parameter was found to be directly predictive of immune-mediated adverse events, the data showed that patients with higher antibody titers reported higher rates of hypersensitivity reactions as compared to those with lower antibody titers.

The identified serious risks of hypersensitivity (including anaphylaxis) will be managed through patient and prescriber labeling (Boxed Warning, and a detailed description in Warnings and Precautions, and Medication Guide) as well as a REMS with ETASU. The identified serious risk of anaphylaxis will be mitigated in the post-marketing setting through a REMS with ETASU which will include prescriber certification, pharmacy certification, and implementation of safe use conditions including patient education and auto-injectable epinephrine availability for use in the event of anaphylaxis. In addition, the label will include a recommendation for product discontinuation if either ≥ 20% blood Phe reduction or a blood Phe concentration below 600 micromol/L is not reached after at least 24 weeks of continuous treatment with 20 mg once daily followed by an additional 16 weeks of continuous treatment with 40 mg once daily. This recommendation aims to minimize the exposure to pegvaliase and the known serious hypersensitivity risks in patients who do not have a favorable therapeutic response. Furthermore, in those patients with an exaggerated therapeutic response (blood Phe concentration falling below 30 micromol/L), the prescribing information will recommend adjustment in protein and Phe intake and/or adjustment of the pegvaliase dose in order to maintain the blood Phe concentration within a clinically acceptable range and above 30 micromol/L.

The remaining uncertainty about the long-term safety risks of chronic use of Palynziq (the current safety database extends to a mean of 2 years with some patients exposed for longer than 4 years), and in particular the long-term clinical effects associated with chronic, high-titer immune responses to the product will be evaluated and further characterized in the post-marketing setting over a longer duration of treatment and in a larger treated patient population. Postmarketing studies, agreed with by the Applicant, will evaluate: 1) the occurrence of serious immune-mediated adverse reactions over long-term treatment with pegvaliase, 2) the impact of immunologic and inflammatory responses associated with chronic pegvaliase treatment (i.e., high-titer, sustained elevations in anti-drug antibodies, low serum complement C3 and C4 levels, high circulating immune complex levels, elevations in C-Reactive Protein and hs-CRP) on safety, including major organ function, and therapeutic responses; and 3) the impact, if any, of prolonged exposure to pegvaliase, given its relatively high PEG content and the fact that the clinical effects of chronic PEG exposure in humans are currently unknown. Knowledge of long-term safety risks as well as the potential long-term effects of the immune and inflammatory responses on the product's efficacy will allow a continuous assessment of the benefits vs. risks associated with this treatment in adults with PKU.

Embryofetal malformations (skeleton, kidneys, lungs, and eyes), and embryofetal toxicity (increased fetal resorptions, reduced fetal weight) were observed in the nonclinical program in the offspring of pregnant rabbits treated with pegvaliase at a dosage which was 7.5 times higher than the maximum recommended human daily dose and in the context of strong signs of maternal toxicity during treatment (marked reductions in maternal weight, food consumption, and animal deaths). While the significance of these nonclinical findings for humans remains unknown, further evaluation in the post-marketing setting seems prudent. Until an explanation can be gleaned from such postmarketing studies to further inform pegvaliase use and labeling, both patients and prescribers should be educated and informed of these potential fetal risks when considering the use of pegvaliase during pregnancy. The prescribing information recommends careful consideration of the benefits and risks of pegvaliase when considering treatment of women with PKU during pregnancy. These potential risks should be considered alongside the known fetal risks associated with untreated or undertreated maternal PKU (intellectual disability, microcephaly, major cardiac malformations). An additional post-approval rabbit study, which the Applicant agreed to conduct, will further evaluate these nonclinical fetal findings by assessing the potential effects of hypophenylalaninemia during pregnancy (and its role in the development of fetal malformations) on pregnant animals and their offspring. Moreover, a post-approval observational study will be instituted to further evaluate safety risks associated with pegvaliase treatment in pregnant women with PKU and their offspring.

In summary, PKU in adulthood is a serious and chronically disabling disease with an unmet medical need for effective treatments, especially in patients with classical PKU. Based on the data presented in this BLA, the review team believes that PALYNZIQ at target doses of 20 mg once daily up to a maximum of 40 mg once daily (depending on individual patient's therapeutic response and tolerability) presents a favorable benefit-risk profile with a REMS and recommends it for approval. Agreement has been reached with the Applicant on the prescribing information, patient labeling, REMS materials, and Post-Marketing Requirements. Remaining uncertainties about long-term safety, safety during pregnancy, and sustainability of the therapeutic response in the context of the product's immunogenicity will be further evaluated in the post-marketing setting through the planned post-approval studies.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Analysis of Condition	• PKU is a rare, autosomal recessive, inborn error of phenylalanine (Phe) metabolism with an incidence in the U.S. of 1 in 10,000 – 15,000 live births. • PKU is typically diagnosed shortly after birth via newborn	• PKU is a rare and serious disease which, when untreated or undertreated, leads to chronic neurologic and psychiatric impairment in adulthood including impaired attention, executive dysfunction,

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	screening detecting high Phe concentrations in blood. PKU presents a spectrum of disease severity ranging from partial enzyme deficiency (mild and moderate PKU) to complete enzyme deficiency (classical PKU). When untreated, patients with classical PKU typically present with blood Phe concentrations >1,200 µmol/L, while those with mild or moderate PKU have blood Phe concentrations between 600 and 1,200 µmol/L. • Without dietary intervention, patients with classical PKU (the most severe form) develop profound and irreversible intellectual disability. Early dietary intervention with protein and Phe intake restriction prevents the profound intellectual disability. However, even with to the availability of dietary management, patients continue to experience suboptimal cognitive outcomes, behavioral and psychiatric disease, executive dysfunction, and impaired attention in their adult years. Clinical outcomes and symptom severity in adults with PKU depend on both current and historical blood Phe control. Clinical outcomes appear to be better when blood Phe is maintained below 600 micromol/L. Reduction in blood Phe concentration is the therapeutic goal in PKU management, and life-long maintenance of blood Phe concentration below 600 micromol/L is a generally accepted treatment goal. Further blood Phe reductions below 360 micromol/L, when possible, are generally encouraged and recommended by the American College of Medical Genetics and Genomics. Management guidelines during pregnancy recommend stricter blood Phe control in the range of 120-360 micromol/L.	depression and anxiety. • Symptoms may be more severe in classical PKU as the blood Phe level tends to be higher than in the mild forms. Hyperphenylalaninemia underlies the neuropathology seen in PKU at all ages. As such, reduction of blood Phe concentrations, which is the therapeutic goal in PKU clinical management, represents an appropriate endpoint for demonstration of efficacy of products intended for treatment of PKU. • Maintenance of blood Phe concentrations below 600 µmol/L appears to confer clinical benefit.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Current Treatment Options	• The PKU diet, which consists of strict restriction of protein and Phe intake, is the standard of care in PKU management in patients of all ages. Dietary management is started as soon the diagnosis is established and should be maintained lifelong. • Kuvan (sapropterin dihydrochloride), which is a cofactor for the PAH enzyme, is approved for patients with PKU who are "responsive" (responsiveness is defined as at least a 30% reduction in blood Phe concentration from baseline after a 4-week treatment trial). Approximately 30% of PKU patients (mostly those with mild or moderate PKU) are Kuvan-responsive, as responsiveness is dependent on genotype and residual PAH enzyme activity. Of patients with classical PKU, only about 10% are Kuvan-responsive.	• The PKU diet is highly-restrictive and difficult to adhere to, particularly for patients with classical PKU in whom stricter dietary management is often needed for metabolic control. As such, the vast majority of adults with PKU are non-compliant with dietary restriction. In addition, the strict dietary protein restriction can contribute to long-term nutritional deficiencies and social acceptance challenges, which contribute to disease burden. • The majority of patients with PKU are not Kuvan-responsive. • There is an unmet need for effective therapies in PKU in the context of the above-described challenges and the absence of effective pharmacologic treatment for the majority of PKU patients, especially those with classical PKU.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Benefit	• The primary efficacy endpoint in the pegvaliase phase 3 trials was reduction of blood Phe concentrations from baseline. In Trial 165-301 (treatment-naïve patients treated through an induction, titration, maintenance regimen), all patients had blood Phe reduced relative to their pretreatment baseline over the duration of the trial, and the response continued into the open-label extension phase of Trial 165-302. • In Trial 165-301, patients who were randomized to and treated with a target dose of 20 mg once daily achieved therapeutic response (defined as blood Phe reduction ≥ 20% from pretreatment baseline or a blood Phe concentration below 600 micromol/L) after a treatment period ranging between 4-24 weeks on the 20 mg once daily target dose; the majority achieved therapeutic response within 4 weeks of treatment on the 20mg dose. • Out of 285 patients enrolled in Trial 165-301, there were 118 with baseline blood Phe > 600 micromol/L who were randomized to and received at least one pegvaliase maintenance dose of 20 mg once daily. In this cohort, 52% achieved a single blood Phe measurement of ≤ 600 micromol/L and 30% achieved blood Phe ≤ 600 micromol/L for 16 consecutive weeks (while in the open-label extension of Trial 165-302). • Pegvaliase efficacy was directly compared against placebo in an 8-week, placebo-controlled, randomized withdrawal design during	• The observed reductions in blood Phe concentration in Trial 165-301 in the adults with PKU who started on and maintained a largely unrestricted dietary protein intake are clinically significant given that the therapeutic goal in PKU management is blood Phe reduction and this is not achieved if dietary protein intake is not restricted. In addition, even though the magnitude of blood Phe reductions in Trial 165-301 varied among patients due to immune-mediated blunting of the therapeutic effect, the majority of patients were able to achieve a predefined therapeutic response (at least 20% reduction in blood Phe concentration or a blood Phe concentration below 600 micromol/L). Overall, adult patients with PKU who were previously in poor metabolic control, as indicated by a pre-treatment blood Phe concentration > 600 micromol/L, were able to achieve blood Phe concentrations below 600 micromol/L, which constitutes a clinically important threshold in PKU management, and indicates improved metabolic control as compared to pre-treatment. Some patients were able to achieve further blood Phe reductions with Phe concentrations within the generally recommended and more stringent range of 120-360 micromol/L, as in the ACMG PKU practice guidelines.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	part 2 of Trial 165-302. Both pegvaliase maintenance doses (20 mg once daily and 40 mg once daily) were efficacious in maintaining blood Phe concentrations for 8 weeks relative to the part 2 study baseline concentrations when compared to matching placebo. Specifically, patients who remained on pegvaliase maintained a stable blood Phe concentration over the 8-week period in contrast to patients who switched to matching placebo whose blood Phe concentration increased over their part 2 baseline and returned to pre-treatment values (primarily in Trial 165-301). The differences in blood Phe concentration between each active and each matching placebo groups at the end of 8 weeks were statistically significant. • In Trial 165-302 part 4 (open-label extension), 32 patients treated with pegvaliase at 20 mg once daily who maintained blood Phe concentrations > 600 micromol/L were further escalated to 40 mg once daily. Of these, 22 (69%) patients experienced further blood Phe reduction to concentrations below 600 micromol/L. • In Trial 165-302, clinical outcomes, such as measures of attention and mood, were assessed over short durations of treatment in a small subset of patients and without a control group, all of which preclude meaningful conclusions on potential treatment effects on such clinical measures. • The degree of blood Phe reduction with pegvaliase treatment was variable among patients and was affected by the immune responses. No single patient characteristic or immune parameter	• Immune responses appeared to blunt the therapeutic effect. • The high rate of drug usage in the pegvaliase trials (>90%) despite the need for daily injections and the reported high frequency of hypersensitivity reactions (see section below) further strengthens the conclusions of treatment benefit.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	was found to be predictive of therapeutic response. • Immunogenicity affected the degree of blood Phe reduction as patients in the highest quartiles for ADA titers showed smaller blood Phe reductions from baseline. • Drug usage in the phase 3 trials was >90%.	
Risk and Risk Management	• Safety was assessed in 285 patients treated with pegvaliase at varying doses through an induction, titration, and maintenance dosing regimen (I/T/M population of Trial 165-301). Additional safety assessments were conducted on all subjects treated with pegvaliase through all dosing regimens in the clinical trials (multi-dose population, N=341) and the safety conclusions were similar in both safety populations. • In the I/T/M population, the median duration of exposure to pegvaliase (at varying doses) was 23.4 months (range 0-59 months) and the median average daily pegvaliase dose was 32 mg/day. • Adverse Events (AEs) seen in the pegvaliase trials were predominantly associated with hypersensitivity; this is not unexpected given that the active ingredient in pegvaliase is a foreign protein. • Serious AEs included hypersensitivity (26 events in 22 patients, 8%), anaphylaxis (37 events in 26 patients, 9%), and elevated CPK (6 events in 5 patients, 2%).	• PKU is a rare disease. The Applicant's safety database included a sufficient number of patients exposed to PALYNZIQ for a sufficient duration to allow for an adequate review of safety and immunogenicity. • Hypersensitivity, including anaphylaxis, was the most frequent serious safety risk identified in the pegvaliase clinical trials. The rate of hypersensitivity events did not appear to depend on the dose used but rather on the time of exposure to the product. The highest rates of hypersensitivity events were seen during induction and titration and the rate decreased with longer exposure. • Product labeling and a Medication Guide will inform physicians and patients of the identified serious safety risks, including the serious risk of anaphylaxis, suggest ways to recognize and mitigate those risks, and help inform appropriate

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	• The most frequent AEs included: hypersensitivity, arthralgia, injection-site reaction, and headache, each of which was reported at least once in at least 50% of treated patients. Causality cannot be fully attributed as there was no placebo control group in Trial 165-301. However, hypersensitivity and injection-site reactions are likely due to product's immunogenicity. • The rate of hypersensitivity AEs, arthralgia, and injection-site reactions was highest during induction and titration (4.68, 7.6, 21.9 events/person-years respectively) and decreased during maintenance (1.5, 1.5, 4 events/person-years respectively). • There was no evidence of an increase in the rate of AEs with higher pegvaliase doses, but rather with the time of exposure. • Similarly, the rate of anaphylaxis was highest during induction and titration and decreased during maintenance. • Most patients who experienced an anaphylaxis event were re-administered pegvaliase at a lower dose and did not experience repeat anaphylaxis episodes. • ~50% of patients who experienced anaphylaxis were treated with auto-injectable epinephrine. There were no deaths or serious sequelae associated with anaphylaxis events. • There is uncertainty as to the impact that the implemented risk mitigation strategies for anaphylaxis (pretreatment with antihistamines and/or antipyretics, presence of a trained "observer") had on the incidence of anaphylaxis during the clinical	benefit/risk decisions for individual patient treatment. • To mitigate the identified safety risks associated with PALYNZIQ treatment, several important recommendations are included in the prescribing information: frequent blood Phe monitoring to assess therapeutic response and discontinuation of treatment if no therapeutic response is achieved; prompt access to auto-injectable epinephrine to treat anaphylaxis; diet modification and/or drug dose adjustment to manage hypophenylalaninemia; and careful benefit-risk assessment and discussion with the healthcare provider when considering PALYNZIQ treatment in pregnant women with PKU (in the context of the product's embryofetal toxicity signal and the fetal toxicity caused by untreated or undertreated hyperphenylalaninemia). • Post-marketing risk mitigation for the serious risk of anaphylaxis will be addressed with a REMS with ETASU program. The REMS will include prescriber certification, pharmacy certification, and implementation of safe use conditions to include patient education and use of auto-injectable epinephrine.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	trials as these risk mitigation strategies were implemented across all treated patients simultaneously during the phase 3 trials. • The most frequent laboratory abnormalities included elevated CRP and hs-CRP, low complement C3 and C4 concentrations, hypophenylalaninemia (blood Phe ≤ 30 micromol/L), elevated CPK, elevated ALT, hematuria, and albuminuria. • Immune-mediated laboratory abnormalities are likely related to pegvaliase and these include elevated CRP, and low C3 and C4 levels. Hypophenylalaninemia is related to pegvaliase and reflects an exaggerated pharmacodynamic response. • There was evidence of maternal toxicity and fetal malformations in the rabbit embryofetal study of pegvaliase without similar findings observed in the rat embryofetal study. The underlying mechanism for the noted fetal malformations is unclear and the potential consequences of PALYNZIQ use during pregnancy in humans is unknown.	• Long-term safety will be assessed in the post-marketing setting via a PMR to conduct a prospective longitudinal observational study that 1) will evaluate the rates of severe immune-mediated adverse reactions, including hypersensitivity reactions, anaphylaxis, and arthralgias, and 2) will assess immunologic and inflammatory responses (anti-drug antibody titers, complement and circulating immune complex concentrations), and their effects on major organs such as the kidneys. • A postmarketing clinical trial will be required to assess the effects of an immune tolerance induction regimen on immune responses to PALYNZIQ and the potential for such a regimen to reduce the risk of severe immune-mediated adverse reactions. • Long-term safety data will be collected in pregnant women treated with pegvaliase and outcomes in their offspring via a post-marketing prospective observational study. • An additional nonclinical study in pregnant rabbits given a diet devoid of Phe will be conducted post-marketing to further evaluate the underlying mechanism for the noted fetal malformations in pregnant rabbits treated with pegvaliase.

1.4 Patient Experience Data

Patient Experience Data Relevant to this Application (check all that apply)

□	The patient experience data that was submitted as part of the application, include:	Section where discussed, if applicable
	□ Clinical outcome assessment (COA) data, such as	[e.g., Section 6.1 Study endpoints]
	□ Patient reported outcome (PRO)	
	□ Observer reported outcome (ObsRO)	
	□ Clinician reported outcome (ClinRO)	
	□ Performance outcome (PerfO)	
	□ Qualitative studies (e.g., individual patient/caregiver interviews, focus group interviews, expert interviews, Delphi Panel, etc.)	
	□ Patient-focused drug development or other stakeholder meeting summary reports	[e.g., Section 2.1 Analysis of Condition]
	□ Observational survey studies designed to capture patient experience data	
	□ Natural history studies	
	□ Patient preference studies (e.g., submitted studies or scientific publications)	
	□ Other: (Please specify)	
X □	**Patient experience data that was not submitted in the application, but was considered in this review.** 1. Meeting between FDA and treated patients in pegvaliase trials, with the National PKU Alliance, on October 31, 2017. 2. FDA public meeting on Patient-Focused Drug Development for Neurological Manifestations of Inborn Errors of Metabolism. June 10, 2014: https://www.fda.gov/downloads/Drugs/NewsEvents/UCM436454.pdf.	

2 Therapeutic Context

2.1 Analysis of Condition

Phenylketonuria (PKU) is an inborn error of phenylalanine (Phe) metabolism characterized by the inability to catabolize Phe, an essential amino acid ingested through the diet, to tyrosine (Tyr) due to genetic deficiency of the enzyme phenylalanine hydroxylase (PAH). PKU is a rare disease with an incidence in the Unites States of 1 in 10,000- 15,000 newborns.[1] PKU is the first inborn error of metabolism included in newborn screening in the United States and is most often diagnosed shortly after birth based on elevated concentrations of Phe in blood.

PKU is caused by biallelic mutations in the *PAH* gene located on chromosome 12q23.2. *PAH* encodes the PAH enzyme that catalyzes the conversion of Phe to Tyr and requires a cofactor, tetrahydrobiopterin (BH4), for its action. Deficiency of PAH activity results in increased systemic levels of Phe and its metabolites, phenyl-acetate and phenyl-lactate. Elevated Phe levels are neurotoxic, although the exact mechanism of this toxicity is not entirely characterized. While the neurologic sequelae of untreated PKU may be due to many proposed factors working in concert (such as abnormal brain myelination, deficient neurotransmitter synthesis, abnormal protein synthesis in the brain, toxicity from Phe metabolites, defective cholesterol synthesis, increased oxidative stress, and altered DNA methylation), elevated Phe concentration is recognized as central in the pathogenesis of the disease.[2] In fact, Phe reduction via dietary management initiated soon after birth has been demonstrated to prevent the devastating neurocognitive consequences of PKU.

Clinical manifestations in untreated PKU patients include intellectual disability, developmental delay, microcephaly, seizures, behavioral problems, and psychiatric disorders. Patients may have a musty or mouse-like odor, light skin pigmentation, and skin rashes if untreated.[3] Elevated maternal blood Phe concentration during early pregnancy is teratogenic and may result in Phe embryopathy. The embryopathic effects of elevated Phe levels during pregnancy in maternal PKU include growth retardation, microcephaly, psychomotor retardation, and congenital heart defects.[4] The best outcomes are achieved with strict control of maternal Phe

[1] https://ghr.nlm.nih.gov/condition/Phenylketonuria#statistics
[2] González MJ, Gassió R, Artuch R, Campistol J. Impaired Neurotransmission in Early-treated Phenylketonuria Patients. Semin Pediatr Neurol. 2016 Nov;23(4):332-340.
[3] https://www.nichd.nih.gov/health/topics/pku/conditioninfo/Pages/symptoms.aspx
[4] American Academy of Pediatrics. Committee on Genetics. Maternal Phenylketonuria. Pediatrics. 2001 Feb;107(2):427-8.

levels before conception and throughout pregnancy.[5]

Disease severity can vary from mild to severe depending on the extent of residual PAH enzyme activity. The most severe form is classical PKU characterized by significantly reduced or absent PAH enzyme activity.[6] Diagnostic evaluation involves plasma amino acid analysis which shows an increased Phe level without increased Tyr (increased phenylalanine:tyrosine ratio).[7] *PAH* mutation testing confirms the diagnosis. [8] As *PAH* gene mutations are characterized, the correlation of these mutations with disease phenotypes are established. For example, the BIOPKU database includes PAH genotypes from over 6,100 affected individuals, and disease phenotype and response or refractivity to Kuvan therapy in almost 3,000 individuals.[9] These emerging genotype-phenotype correlations may provide insights into disease severity prediction, enable appropriate genetic counseling, help identify suitable treatment options, and provide accurate information of disease prognosis; in addition, improved knowledge of how genotype affects disease manifestations may provide added benefits to affected patients such as better insurance coverage for treatments with resultant decreased unnecessary costs as well as enhanced knowledge on the disease on a population level[10]

Early identification of PKU with newborn metabolic screening and early treatment with Phe-restricted diet significantly improved cognitive outcomes in children. The American College of Medical Genetics and Genomics (ACMG) practice guidelines state that "initiation of treatment for PKU should be undertaken as early as possible, preferably within the first week of life with a goal of having blood Phe in the treatment range within the first 2 weeks of life."[11] Dietary discontinuation before 8 years of age is associated with poorer performance on IQ measures, although the consequences of dietary discontinuation at older ages are less clear.[12] A systematic literature review and meta-analysis by Waisbren SE et al. found significant proportional correlations "during critical periods (from 0 to 12 years of age) for early-treated

[5] American Academy of Pediatrics. Committee on Genetics. Maternal phenylketonuria. Pediatrics. 2001 Feb;107(2):427-8.
[6] https://ghr.nlm.nih.gov/condition/phenylketonuria#genes
[7] "Newborn Screening ACT Sheet [Increased Phenylalanine] Phenylketonuria (PKU)" *American College of Medical Genetics, 2010*.
[8] "Newborn Screening ACT Sheet [Increased Phenylalanine] Phenylketonuria (PKU)" *American College of Medical Genetics, 2010*.
[9] Camp KM, Parisi MA, Acosta PB et al. Phenylketonuria Scientific Review Conference: a state of the science and future research needs. Mol Genet Metab. 2014 Jun;112(2):87-122.
[10] Camp KM, Parisi MA, Acosta PB et al. Phenylketonuria Scientific Review Conference: a state of the science and future research needs. Mol Genet Metab. 2014 Jun;112(2):87-122.
[11] Vockley J, Andersson HC, Antshel KM et al. Phenylalanine hydroxylase deficiency: diagnosis and management guideline. *Genet Med*. 2014 Feb;16(2):188-200.
[12] Howell RR. National Institutes of Health Consensus Development Conference Statement: Phenylketonuria: screening and management, October 16–18, 2000. Pediatrics 2001;108(4):972–982.

patients with PKU…where each 100 micromol/L increase in blood Phe predicted a 1.3- to 3.1-point reduction in IQ. Similar significant correlations were observed between IQ and mean lifetime Phe level for early-treated patients…"[13] In addition, early treated children and adolescents with PKU have a higher frequency of attention deficit hyperactivity disorder, decreased autonomy, and school problems compared with either healthy controls or children with other chronic conditions.[14]

Liberalization of the Phe-restricted diet was historically part of PKU management. However, the guidelines have changed to recommend lifelong treatment with a Phe-restricted diet with a goal of maintaining blood Phe within a target therapeutic range. This is based on growing evidence that Phe control throughout life improves neurocognitive and psychiatric functioning.[15] A longitudinal study over 32 years followed infants who were treated with a Phe-restricted diet to age 6 years and then randomized to continue or discontinue dietary treatment. Koch R et al. concluded that the subjects who discontinued the diet had an increased rate of eczema, asthma, mental disorders, headache, hyperactivity and hypoactivity compared to those subjects who maintained the diet. They also concluded that lower intellectual and achievement test scores were associated with dietary discontinuation and with higher childhood and adult blood Phe concentrations.[16] Other studies have found that if the diet is liberalized after 12 years of age, IQ can remain stable but other functions decline. "Adults who have abandoned the Phe-restricted diet tend to have a reduced attention span, slow information-processing abilities, and slow motor reaction time."[17] However, there are some discrepancies in the literature and other studies found no correlation with Phe level and executive functioning.[18]

While PKU patients show a spectrum of clinical manifestations depending on individual disease severity, early or late dietary treatment, and blood Phe control over time, the therapeutic goal in PKU management is blood Phe reduction. Blood Phe concentration is the fundamental measure of clinical outcomes and is monitored routinely and frequently during treatment and throughout a patient's lifetime. Based on ACMG practice guidelines, treatment of PKU must be

[13] Waisbren SE, Noel K, Fahrbach K et al. Phenylalanine blood levels and clinical outcomes in Phenylketonuria: a systematic literature review and meta-analysis. Mol Genet Metab. 2007 Sep-Oct;92(1-2):63-70.
[14] Mitchell JJ, Trakadis YJ, Scriver CR. Phenylalanine hydroxylase deficiency. Genet Med. 2011 Aug;13(8):697-707.
[15] Vockley J, Andersson HC, Antshel KM et al. Phenylalanine hydroxylase deficiency: diagnosis and management guideline. *Genet Med*. 2014 Feb;16(2):188-200.
[16] Koch R, Burton B, Hoganson G et al. Phenylketonuria in adulthood: a collaborative study. J Inherit Metab Dis. 2002 Sep;25(5):333-46.
[17] Mitchell JJ, Trakadis YJ, Scriver CR. Phenylalanine hydroxylase deficiency. Genet Med. 2011 Aug;13(8):697-707.
[18] Christ SE, Huijbregts SC, de Sonneville LM, White DA. Executive function in early-treated phenylketonuria: profile and underlying mechanisms. Mol Genet Metab. 2010;99 Suppl 1:S22-32.

lifelong with a goal of maintaining blood Phe in the range of 120–360 µmol/l. [19] Treatment options are limited to dietary protein and Phe restriction and Kuvan (sapropterin dihydrochloride), as an adjunct to dietary restriction, in those who are Kuvan-responsive (~30% of PKU patients), and discussed in more detail below.

Published in 2015 in Molecular Genetics and Metabolism Reports, the National PKU Alliance (NPKUA) conducted a survey of its membership to assess current health status and interest in new PKU treatments. Of the 625 survey respondents, 46.7% reported blood Phe within 120 – 360 micromol/L. 51.7% of respondents reported having difficulty managing their PKU, including the maintenance of a Phe-restricted diet. Respondents "reported that blood Phe reduction, improved attention, and improved executive function were the most important potential benefits of new treatments." The NPKUA survey results showed that "individuals with PKU desire new treatments that would allow them to increase their intake of natural protein, discontinue or reduce their intake of medical foods (medical formula and foods modified to be low in protein), improve their mental health (including a reduction in depression and anxiety), and a reduction of their blood Phe concentrations."[20]

2.2 Analysis of Current Treatment Options

The current standard of care for PKU management is strict restriction of dietary protein and Phe intake (PKU diet). This involves significantly decreasing the intake of natural protein and replacing it with medical food, which is often a mixture of essential and non-essential amino acids without Phe. The medical food needs supplementation of calories, vitamins, and nutrients necessary for optimal growth and development in order to prevent nutritional deficiencies that arise from a lack of normal natural dietary protein intake. The PKU diet involves a variety of modified, low-protein foods which are specially prepared and often costly. As dietary requirements change over time based on a patient's age, growth rate, and activity level, close monitoring of blood Phe and overall health is an essential part of PKU management and is performed by a team comprised of a clinical/biochemical geneticist and a metabolic dietitian.[21]

Adherence to a strict Phe-restricted diet is challenging for many adults with PKU. These reasons include limited dietary choices, poor palatability of medical foods, and high cost. In addition, this population often has problems with executive function which may lead to

[19] Vockley J, Andersson HC, Antshel KM et al. Phenylalanine hydroxylase deficiency: diagnosis and management guideline. *Genet Med*. 2014 Feb;16(2):188-200.
[20] Brown CS, Lichter-Konecki U. Phenylketonuria (PKU): A problem solved? Mol Genet Metab Rep. 2015 Dec 29;6:8-12.
[21] Vockley J, Andersson HC, Antshel KM et al. Phenylalanine hydroxylase deficiency: diagnosis and management guideline. *Genet Med*. 2014 Feb;16(2):188-200.

difficulty calculating and tracking protein intake and other nutritional requirements. Finally, patients with PKU may have psychosocial issues related to differentiation in the social setting due to diet restriction and not being able to eat with others or in certain places such as restaurants.

The only currently approved treatment for PKU is Kuvan (sapropterin dihydrochloride). Kuvan was approved by the FDA in 2007 and is indicated to reduce blood Phe levels in patients over 1 month of age with hyperphenylalaninemia due to tetrahydrobiopterin-(BH4-) responsive PKU in conjunction with a Phe-restricted diet.[22] Sapropterin is a synthetic form of BH4, the essential cofactor of the PAH enzyme. Treatment with BH4 activates the PAH enzyme, improves the normal oxidative metabolism of Phe, and decreases Phe levels in those patients who have some residual enzyme activity.[23] As stated in the ACMG practice guidelines, "patients with mild PAH deficiency are more likely to respond [to sapropterin] because some stable protein is required for sapropterin to function; nonetheless, responsive patients are identified even among those with complete PAH deficiency…Therefore, every PAH-deficient patient should be offered a trial of sapropterin therapy to assess responsiveness except those with two null mutations in *trans*."[24] Patients with PKU are considered sapropterin-responsive if they achieve at least a 30% reduction in blood Phe concentration from their pre-treatment baseline after 1 month of treatment at the highest approved dose (20 mg/kg/day). Approximately 25-50% of PKU patients are considered sapropterin-responsive.[25]

Dietary supplementation with large neutral amino acids (LNAA) is an experimental treatment for PKU. The mechanism of action of LNAA is blocking the uptake of Phe from the intestine and at the blood-brain barrier. A single clinical trial showed reduction of blood Phe by approximately 40% following dietary substitution of low Phe medical food with LNAA, but larger trials are needed to evaluate efficacy and safety.[26] LNAA is not considered available therapy from a regulatory perspective, as it is not an approved drug.

Thus, for most patients with PKU, particularly those with classical PKU (with complete enzyme deficiency) there is an unmet medical need as diet is the only available treatment option.

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[22] https://www.accessdata.fda.gov/drugsatfda_docs/label/2016/022181s014lbl.pdf. Labeling-Package Insert dated 6/13/2016.
[23] https://www.accessdata.fda.gov/drugsatfda_docs/label/2016/022181s014lbl.pdf. Labeling-Package Insert dated 6/13/2016.
[24] Vockley J, Andersson HC, Antshel KM et al. Phenylalanine hydroxylase deficiency: diagnosis and management guideline. *Genet Med*. 2014 Feb;16(2):188-200.
[25] Vockley J, Andersson HC, Antshel KM et al. Phenylalanine hydroxylase deficiency: diagnosis and management guideline. *Genet Med*. 2014 Feb;16(2):188-200.
[26] Vockley J, Andersson HC, Antshel KM et al. Phenylalanine hydroxylase deficiency: diagnosis and management guideline. *Genet Med*. 2014 Feb;16(2):188-200.

Therapies may be individualized based on feasibility and response, with the primary goal of reducing the blood Phe level but also improving quality of life.

3 Regulatory Background

3.1 U.S. Regulatory Actions and Marketing History

Pegvaliase is a new molecular entity and is not currently marketed outside the U.S.

3.2 Summary of Presubmission/Submission Regulatory Activity

- All pre-submission activities took place under IND 076269. The Table below summarizes the most important pre-submission meetings between FDA and the Applicant and highlights major discussion points throughout the clinical development program.

Table 1: Summary of Presubmission/Submission Regulatory Activity

Date	Meeting type	Highlights
3/8/1995		Orphan Drug Designation granted
8/2/2011	Type C, Clinical	FDA agreed "that reduction of blood phenylalanine level is an acceptable primary efficacy measure for studies in adult PKU patients."
11/22/2011		Fast Track Designation granted
1/29/2013	Type B, End-of-Phase 2 (EOP2)	FDA states the need to define a responder and provide scientific (data-based) justification for the definitions. Also, FDA states that primary endpoints should be clinical endpoints.
9/30/2014	Type C, Clinical	FDA recommends "that [you] add additional clinical measures of attention and mood to the ongoing studies to support the clinical relevance of change in Phe in adults."
8/18/2015	Type C, Biostatistics	Discussion of statistical analysis plans and PK analysis for the Phase 3 studies with regards to pooling the active and placebo groups. FDA indicated that "the high incidence of anti-drug antibodies (ADAs) observed in the BMN 165 (pegvaliase) program is of concern not only from an efficacy perspective but also from a safety standpoint." "FDA

		re-affirmed their reservation regarding the choice of a 20% reduction in plasma Phe as a criterion for identifying responders at the end of the Study 301 who will later be enrolled in Study 302, and questioned how this definition will be informative for labeling the product."
3/15/2016	Type C, Product Quality, Immunogenicity	FDA expressed concerns "that a high daily dosing regimen and high prevalence of ADAs will lead to the formation of immune complexes and subsequent complement activation…" and expressed the need for validated and sensitive methods for antibody monitoring. Also, FDA noted the need to monitor increasing antibody titers and antibody cross reactivity.
11/22/2016	Type C, Immunogenicity	Agreement with the Sponsor to submit IgM-CIC and IgG-CIC validation reports and clinical data from all Phase 3 treated patients in the initial BLA submission and for IgG4 antibody validation report to be submitted with the 120-day safety update. Also, concerns from clinical pharmacology that "the total pegvaliase concentrations measured using the current PK assay are not reflective of the active drug concentrations."
5/31/2017	Type C, Clinical and Clinical Outcome Assessments (COA)	Discussion on an observational study to inform development and selection of appropriate COA instruments for use in future trials in an adult PKU population.

4 Significant Issues from Other Review Disciplines Pertinent to Clinical Conclusions on Efficacy and Safety

4.1 Office of Scientific Investigations (OSI)

Inspections for this BLA consisted of inspections of three clinical investigator (CI) sites and the sponsor BioMarin Pharmaceutical, Inc. The data generated by these sites and the applicant are deemed acceptable in support of the application. See separate review by Dr. Susan Leibenhaut, Dr. Susan Thompson, and Dr. Kassa Ayalew (finalized on February 9, 2018; Reference ID: 4219825).

4.2 Product Quality

The Office of Pharmaceutical Quality (OPQ), CDER, recommends approval of BLA 761079 for pegvaliase manufactured by BioMarin Pharmaceutical Inc. The data submitted in this application are adequate to support the conclusion that the manufacture of pegvaliase is well-controlled and leads to a product that is pure and potent. It is recommended that this product be approved for human use under conditions specified in the package insert.

Note the BLA was initially granted a priority review (with PDUFA goal date of February 28, 2018), but a major amendment related to drug product sterility assurance submitted on December 15, 2017, led to an extension of the PDUFA goal date to May 28, 2018. The drug product manufacturing process consists of (b) (4) BioMarin agreed to implement (b) (4) as a postmarketing commitment; the change will reduce unnecessary manipulation of the sterile drug product. In addition, BioMarin will evaluate product quality before and after shipping of the prefilled syringes as a postmarketing commitment.

The drug substance manufacturing process consists of (b) (4). Due to changes implemented to the (b) (4) process, BioMarin re-validated the (b) (4) during the extended review cycle. There are postmarketing commitments (PMCs) related to drug substance manufacturing, including evaluating the impact of removal of kanamycin from the fermentation process and evaluating product quality before and after shipping of formulated bulk drug (b) (4). The analytical methods for release and stability testing are validated for specificity, precision, repeatability, linearity, accuracy, and robustness. There is one PMC related to revising the enzyme kinetic assay. For a list of all postmarketing studies, refer to the final approval letter and the finalized postmarketing study templates.

The manufacturing facilities for drug substance (BioMarin, Novato, CA) and drug product (Cook, Bloomington, IN) are recommended for approval.

Reviews with specific details regarding product quality, immunogenicity assays, and labeling (by OBP, DBRR-IV), microbiology (by DMA, Branch IV), facilities (by DIA, Branch I), and device (by CDRH) are located as separate documents finalized in the CDER Informatics Platform/Panorama or DARRTS.

4.3 Clinical Microbiology

Not applicable.

4.4 Devices and Companion Diagnostic Issues

CDRH recommends approval based on the review of the device constituent of this 21 CFR Part 3 combination product. Review of this information found that there are sufficient verification activities for the safety and functionality of the device constituent portion of the combination product to recommend approval. See separate review by Dr. Rong Guo, Dr. John McMichael, and Dr. Alan Stevens (finalized on February 12, 2018; Reference ID: 4220083).

5 Nonclinical Pharmacology/Toxicology

This represents a high-level summary of the primary review by Dr. Fang Cai (finalized on March 6, 2018; Reference ID: 4230623), and the discussion of key/notable safety issues.

To support the intended clinical use, the Sponsor conducted studies in pharmacodynamics (PD), safety pharmacology, general toxicology, and developmental and reproductive toxicology in mice, rats, monkeys, and rabbits. The PD studies were conducted in BTBRPahenu2 (ENU2) mice, an animal model of PKU.

Pegvaliase at SC doses of 0.2 to 120 mg/kg given once weekly or three times per week in ENU2 mice produced a dose- and dosing frequency-dependent decrease in blood Phe levels associated with increased weight gain, lifespan, and reproductive capabilities. Pegvaliase at 10 to 20 mg/kg/week for 12 weeks increased the number of tyrosine hydroxylase-expressing neurons in hypothalamus, midbrain, and dorsomedial hypothalamic nucleus, although the number of neurons was still lower than that of wild type mice. An interim attenuated PD response due to anti-pegvaliase antibody formation was usually seen between weeks 3-7 of treatment. Increasing anti-pegvaliase titers were observed throughout the repeat dose studies. A single SC dose up to 125 mg/kg pegvaliase had no effects on CNS or respiratory functions in rats. No cardiovascular effects were observed at a single dose of 10 mg/kg in monkeys.

After a single SC administration in rats and monkeys, first-order absorption and monophasic elimination were observed, with no apparent sex-related differences observed. The bioavailability of pegvaliase after SC injection in the rat was low (13% to 17%). Following administration of repeated doses via twice weekly SC injection in the two species, plasma drug concentrations increased after week 1 in rats and week 2 in monkeys. Thereafter, pegvaliase levels were decreased, likely due to the formation of neutralizing or eliminating antibodies against pegvaliase. Plasma levels of pegvaliase increased in a dose-dependent manner with little to no accumulation. The T_{max} for the rat and monkey with single SC injection ranged from 12 to 120 hours and 48 to 108 hours, respectively, indicating a slow rate of absorption from the injection site into circulation. The apparent terminal half-life ($t_{1/2}$) ranged from 33 to 53 hours and 51 to 92 hours in rats and monkeys, respectively, demonstrating a slow rate of elimination. The long terminal $t_{1/2}$ observed in the rat with SC dosing was consistent with the long half-life observed with IV dosing (25 to 46 hours).

In a 4-week SC toxicity study, SD rats were treated with pegvaliase at doses of 1, 8, and 25 mg/kg twice weekly. Pegvaliase had no adverse effects on body weight, food consumption, hematology, clinical chemistry, or urinalysis. Pegvaliase at ≥ 8 mg/kg produced minimal vacuolation in reticuloendothelial cells in spleen and fibrosis at the injection site. None of these findings were considered adverse. Therefore, the NOAEL was 25 mg/kg. Overall, there was a

dose proportional increase in systemic exposure to pegvaliase. No accumulation of pegvaliase was observed following repeated doses. Anti-drug antibodies (IgG) were detected in some animals, and the incidence and titers were not dose-dependent.

In a 26-week SC toxicity study with a 17-week interim sacrifice, SD rats were treated with pegvaliase at doses of 1, 8, or 25 mg/kg/day twice weekly. Slight to moderate decreases in body weight, body weight gain, and food consumption were observed in the 25 mg/kg group. Pegvaliase at 8 and/or 25 mg/kg produced slight to moderate decreases in triglycerides, ALT, ALP, and urine pH. Following the 17- and 26-week treatment periods, similar treatment-related histopathological changes were observed in the kidney, spleen, liver, testes, adrenal gland, mesenteric and mandibular lymph nodes, and SC injection sites in the 8 and/or 25 mg/kg groups. The histopathological changes included focal to multifocal areas of vacuolation/hypertrophy of renal tubule cells, increased vacuolation in histiocytic cells in the adrenal cortex, liver, spleen, mesenteric and mandibular lymph node, and testes. The incidence and severity of these changes increased with dose level and treatment duration. At the end of the 12-week recovery phase, all of these changes persisted, but with less severity. Anti-pegvaliase antibodies did not bind to the cytoplasmic vacuoles in renal tubular epithelium or in histiocytic cells in spleen and lymph nodes. However, PEG was detected (via immunohistochemistry (IHC) using anti-PEG antibodies) in Kupffer cells in liver sinusoids, sinus histiocytes in the mesenteric lymph nodes, and in renal epithelial cells. Kidney was considered as the target organ of toxicity, based on the incidence of vacuolation/ hypertrophy of renal tubule cells at ≥ 8 mg/kg, which failed to reverse after the 12-week recovery period. The NOAEL was 1 mg/kg twice weekly. Systemic exposure to pegvaliase generally increased in a dose-proportional manner. No accumulation of pegvaliase occurred following repeated doses. In general, females had higher pegvaliase C_{max} and AUC_{0-t} values than males. Anti-drug antibodies (IgG) were detected in most animals. The vacuolation observed in multiple organs was only associated with slight to moderate decreases in liver enzymes (AST, ALP) and urine pH. Thus, the clinical importance or relevance is unclear.

In a 4-week SC toxicity study, monkeys were treated with pegvaliase at doses of 0.01, 0.1, or 1 mg/kg twice weekly. Pegvaliase at ≥ 0.1 mg/kg caused minimal to slight vascular degeneration, mainly in the medium-sized, muscular arteries of the following organs: lung, stomach, gallbladder, kidney, colon, pancreas, spleen, and prostate. The vascular degeneration was reversible. No IHC was conducted to investigate whether the vascular degeneration was related to immune complexes. The target organs of toxicity were arteries and injection sites. The NOAEL was 0.01 mg/kg based on degeneration of arteries at 0.1 mg/kg and higher.

In a 39-week SC toxicity study, monkeys were treated with pegvaliase at doses of 0.01, 0.1, 1, 3, or 7/5/3 mg/kg twice weekly. The high dose of 7 or 5 mg/kg was not tolerated based on the severe reduction in food consumption, significant body weight loss and/or hypoactivity. Thus, the dose was reduced to 3 mg/kg. Pegvaliase at ≥ 3 mg/kg produced systemic arteritis involving

small arteries and arterioles in a wide range of organs and tissues (kidney, urinary bladder, pancreas, gallbladder, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, lung, heart, sciatic nerve, lacrimal gland, mandibular lymph node, epididymis, seminal vesicle, ovary, uterus, cervix, and vagina) and in SC injection sites. No arterial inflammation was observed at the end of the recovery period. Immune-complex material was detected in affected vessels in the organs examined (heart, kidney, and liver) in animals treated at ≥ 3 mg/kg. Pegvaliase and/or its PEG moiety identified by IHC were detected in intravascular proteinaceous material, endothelium, interstitial proteinaceous material, and mononuclear cells in the liver and kidney at ≥ 3 mg/kg. There were no treatment-related changes in hematology or clinical chemistry. The target organs of toxicity were arteries in multiple organs and bone marrow (increased lymphoid nodules). The NOAEL was considered to be 1 mg/kg twice weekly. The drug-induced systemic arteritis was likely related to the vascular accumulation of immune-complex material generated from the immunogenic response to pegvaliase, since immune-complex was detected in the affected vessels. Systemic arteritis was not associated with adverse changes in hematology, clinical chemistry, or histopathology in liver, kidney or other organs. Arterial inflammation was reversible after cessation of treatment. Thus, the clinical importance and translatability to humans of systemic arteritis observed in the monkeys is unknown.

In a combined fertility and embryo-fetal development (segment 1/2) study, male and female rats were treated with pegvaliase at doses of 0, 2, 8 or 20 mg/kg/day via SC injection before cohabitation, with continuation of dosing through mating, implantation and closure of the hard palate GD 17. At 20 mg/kg/day, pegvaliase produced maternal or paternal toxicities (e.g. slight to moderate decreases in body weight, body weight gain, and food consumption). Pegvaliase at ≥ 8 mg/kg/day significantly reduced the number of corpora lutea and implantations, litter size (20 mg/kg/day only), live fetuses (20 mg/kg/day only), and fetal weights (20 mg/kg/day only). Pegvaliase at ≥ 8 mg/kg/day significantly increased fetuses with alterations, which were limited to variations such as cervical ribs, bifid centra of lumbar and thoracic vertebrae, and incomplete ossification of squamosal bones, frontal bones, lumbar vertebra arch, and ribs. Systemic exposure to pegvaliase was detected in fetuses from the 20 mg/kg group. Pegvaliase produced a dose-dependent decrease in blood Phe levels in both sexes. The Phe level was below the low limit of quantification (1 µM) at 20 mg/kg/day. The NOAEL for male fertility was 20 mg/kg/day, and the NOAEL for female fertility was 8 mg/kg/day based on the decrease in corpora lutea at 20 mg/kg/day. The NOAEL for embryo-fetal development was 8 mg/kg/day, based on the reduced fetal weights at 20 mg/kg/day.

In an embryo-fetal development study in rabbits, pregnant females were treated with pegvaliase at doses of 2 or 5 mg/kg daily (SC) using a divided dosing regimen (GD 7 to 12, GD 11 to 16, or GD 15 to 20), to provide the most consistent level of plasma exposure to pegvaliase throughout organogenesis (GD 7 to 20). The rationale for this study design was supported by toxicokinetic data from the dose-ranging study in pregnant rabbits, which demonstrated

profound changes in AUC and C_{max} after a few days of dosing (values were decreased with 2 mg/kg/day, and increased with 5 mg/kg/day). The changes in TK parameters with repeated dosing were likely due to the effect of anti-drug antibodies. SC administration of 5 mg/kg/day produced a high incidence of external malformations of the head, body, and limbs, and multiple malformations in visceral organs and all regions of the skeletal system (e.g. > 50% incidence of shortened limbs among fetuses and litters). Although the dose which produced malformations also caused clear signs of maternal toxicity (e.g. abortion and premature death in 8% of rabbits, marked impairment of weight gain and food consumption in the surviving females), the malformations are not considered to be secondary to maternal toxicity based on their severity and high incidence. Other adverse effects observed at 5 mg/mg/day included significant increases in late resorptions, post-implantation loss, and the number of does with any resorptions. Reductions in male and female fetal weight and the number live male fetuses were also observed. The observed reduction in maternal weight gain during days 7-29 of gestation was 23% and 59% in the 2 and 5 mg/kg/day groups, respectively. These values represent the mean reduction observed in the three treatment periods used in this study (i.e. GD 7-12, 11-16 and 15-20). During treatment (days 7-20 of gestation), plasma Phe levels in the 2 and 5 mg/kg/day groups were decreased by an average of 90% and 99%, respectively. The NOAEL for embryo-fetal developmental toxicity was 2 mg/kg/day. The maternal NOAEL was not identified (< 2 mg/kg/day), due to the reductions in weight gain and food consumption in the 2 mg/kg/day group.

In a pre-/postnatal development study in rats, pegvaliase at 20 mg/kg/day produced significant decreases in maternal body weight gain (up to 25.8%) and food consumption (↓up to 33.4%). At 20 mg/kg/day, pegvaliase significantly reduced viability index (↓15.5%), lactation index (↓9%), pup survival during postpartum days 1-4 (↓up to 28.7%), and pup weight during lactation (↓up to 28.3%). The offspring from the 20 mg/kg/day group had significant decreases in body weight, body weight gain, and food consumption (males only), and showed a delay in sexual maturation. No pegvaliase was detected in the offspring. However, pegvaliase was detected in milk at all doses (2, 8, and 20 mg/kg/day). There was a dose-dependent reduction in maternal plasma phenylalanine level. The apparent NOAEL for developmental effects was 8 mg/kg/day. However, this conclusion is only preliminary, given that this study lacked sufficient testing to provide an adequate evaluation of postnatal development. Learning and memory in the offspring (F1 generation) were evaluated through the Passive Avoidance and M-Maze Water Maze tests, which showed no effects of pegvaliase. However, no other tests were conducted to evaluate behavior, motor activity, sensory or sensorimotor functions, or reflex development. Furthermore, this study was deficient in the evaluation of early landmarks of physical development. The adverse effects in offspring at 20 mg/kg/day may have been secondary to maternal toxicity. The NOAEL for maternal toxicity was 8 mg/kg/day. This study should be repeated with the inclusion of tests to evaluate the effects on behavior, motor activity, sensory or sensorimotor functions, and reflex development, and an adequate evaluation of early physical development.

Vacuolation in multiple organs in the repeat-dose rat toxicity studies (with sacrifices at 4, 17 and 26 weeks) at ≥ 8 mg/kg was further evaluated in selected organs (adrenal, kidney, liver, mesenteric lymph node, mandibular lymph node, spleen, and testis) by a validated IHC method using anti-pegvaliase and anti-PEG antibodies.

Minimal to mild binding of anti-pegvaliase antibodies was present in kidney, and the incidence and severity was related to dose and duration of treatment. Binding of anti-pegvaliase antibodies in blood vessels (endothelium and intraluminal contents) was observed in glomeruli and in interstitial vessels in cortex, medulla, and papilla of kidney. The vascular staining most likely represented circulating pegvaliase within the lumen of blood vessels. Binding in epithelial cells was cytoplasmic and present only in the renal papilla in some animals. Binding was present in blood vessels and sinusoids in liver (weeks 17 and 26) and in follicular lymphocytes in some spleen samples (week 4). Anti-pegvaliase antibodies did not bind to vacuoles in renal tubular epithelium, histiocytic cells in spleen, or in mandibular and mesenteric lymph nodes.

Cytoplasmic anti-PEG staining was mainly present in vascular spaces (plasma/endothelium) in kidney, adrenal gland, spleen, liver, lymph node (mesenteric mandibular) and testes. Cytoplasmic anti-PEG staining in non-vascular spaces was observed in Kupffer cells in liver sinusoids, sinus histiocytes (lymph node), and renal tubular cells (only in rats administered 25 mg/kg through study week 17 for staining of renal tubules). The intensity of staining was dose-dependent.

The presence of positive staining by anti-pegvaliase antibodies in renal tubular epithelium and in infiltrating or fixed macrophages (histiocytes) in adrenal gland, liver, lymph node (mandibular and mesenteric), and spleen was likely indicative of the clearance mechanisms for PEG.

Leachables, including organic and elemental leachables, were detected in the drug product (pre-filled syringe) stored at 2-8°C for 24 months or at 25°C for 6 months (accelerated conditions). The toxicology risks of these leachables and elements were assessed. The potential exposure to leachables at the maximum recommended daily dose of 40 mg pegvaliase does not present any safety concerns (see section 2.5 and Appendix of the primary review by Dr. Fang Cai [DARRTS 3-6-2018] for details).

Reviewer Comments:

1. There are no nonclinical issues which preclude the approval of Palynziq injection.

2. Pegvaliase is a highly PEGylated homotetrameric protein. PEGylation of this product involves the conjugation of linear 20 kDa N-hydroxysuccinimide (NHS)-methoxypolyethylene glycol (NHS-PEG) with recombinant phenylalanine ammonia lyase

(rAvPAL). The current specification for the degree of PEGylation at release is (b) (4) PEGs/monomer.

In the amendment received on 03/23/2018, the Sponsor provided information on the amount of PEG delivered with the proposed dose levels, as shown in the Sponsor's table below.

Pegvaliase dose (mg rAvPAL)	Average mg amount of PEG
2.5	7.3
10	29
20	58
40	117

The proposed maintenance dose levels for pegvaliase are 20 and 40 mg rAvPAL/ day, which indicates that maintenance therapy will deliver an average of either 58 or 117 mg/day of PEG (20 kDa), respectively, via SC injection. This dose of high molecular weight PEG appears to exceed that of other approved therapeutic PEGylated proteins, based on comparison to a small number of approved PEGylated proteins, using a dose comparison normalized to reflect the average daily administration of PEG. However, it is emphasized that a comprehensive review of approved PEGylated proteins was not conducted to provide comparative data for pegvaliase.

The general toxicology studies in rats and monkeys demonstrated evidence of PEG accumulation in rats only. However, the absence of detectable PEG accumulation or vacuolation in monkeys can be attributed to the use of lower dose levels, necessitated by the intolerance to higher dose levels. Rats showed a dose-dependent vacuolation in the 4-week and 26-week SC toxicity studies at ≥ 8mg/kg pegvaliase twice weekly (0.2 times the human steady state AUC for pegvaliase at the maximum recommended daily dose in humans). Vacuolation of histiocytes was observed in liver, spleen, testes, adrenal cortex, mesenteric lymph node, and mandibular lymph node, whereas vacuolation in kidney occurred in tubule cells. No clear evidence of target organ toxicity was observed in the organs where vacuolation occurred, as determined by clinical chemistry/urinalysis and histopathological examination. Regardless, the Sponsor considered the kidney as a target organ of toxicity in the 26-week toxicity study in rats, based on the incidence of vacuolation/hypertrophy of renal tubule cells at ≥ 8 mg/kg, which failed to reverse after the 12-week recovery period. IHC using anti-PEG antibodies confirmed the presence of PEG in Kupffer cells in liver sinusoids, sinus histiocytes in the mesenteric lymph nodes, and in renal tubular epithelial cells. No vacuolation was observed at 1 mg/kg. The extent of vacuolation

observed in a 17-week interim sacrifice group in the 26-week rat study was similar to that in the terminal (main study) group. Therefore, it appears that steady state accumulation of PEG was achieved.

In the amendment received on 03/27/2018, the Sponsor confirmed that the amount of PEGylation in the drug lots (8 – 12 PEGs/monomer) used in nonclinical studies was similar to that of the commercial product. The Sponsor also clarified that the stated dose levels in nonclinical studies were based on the amount of protein (rAvPAL). Therefore, the dose levels of PEG in the 26-week rat study were calculated to be 0.83, 6.6, and 20.7 mg/kg/day (the daily doses were extrapolated from the twice weekly dosing frequency used in this study). The maximum recommended human dose of 40 mg/day pegvaliase will deliver a PEG dose of about 117 mg (1.95 mg/kg/day PEG based on an assumed body weight of 60 kg). Therefore, the NOEL for vacuolation/PEG accumulation in rats (0.83 mg/kg/day PEG) is only 0.4 times the maximum PEG dose in humans, based on a mg/kg comparison. The LOEL (6.6 mg/kg/day PEG) was 3.4 times the maximum PEG dose in humans.

It is likely that vacuolation in histiocytes of multiple organs was related to PEG accumulation, an event that has been reported with other PEGylated therapeutic proteins. The greatest concern about potential toxicity from PEG appears to be the reported PEG accumulation in choroid plexus, synovium, and choroid of the eye, none of which were observed in rats treated with pegvaliase. It is noteworthy that the Sponsor included a dedicated examination of the choroid plexus in the general toxicology studies in rats and monkeys. Specifically, all H&E stained brain sections from the 26-week rat and 39-week monkey toxicity studies were evaluated by light microscopy at 40x and 100x magnification for cytoplasmic vacuolation of ependymal cells of the choroid plexus, which line the brain ventricles. This procedure was done to comply with the recommendations established in a publication issued by the European Medicines Agency (CHMP Safety Working Party's response to the PDCO regarding the use of PEGylated drug products in the pediatric population). Vacuolation was not observed in choroid plexus.

A major consideration in the safety assessment of PEG accumulation is the known impact of the PEG molecular weight on both the tendency of PEG to accumulate, and the tissues in which it accumulates. PEGs with a MW of $\leq$ 20 kDa primarily accumulate in tissue macrophages (histiocytes) and renal tubular epithelial cells. In contrast, PEGs of $\geq$ 40 kDa are known to accumulate in the choroid plexus, synovium, and choroid of the eye. The PEG molecular weight in pegvaliase is 20 kDa, and the tissue pattern of vacuolation and IHC-confirmed PEG accumulation in rats treated with pegvaliase is consistent with the known distribution of PEG $\leq$ 20 kDa. Although the accumulation of PEG ($\leq$ 20 kDa) in histiocytes suggests a theoretical concern about impaired histiocyte function resulting in diminished immune responses, we are not aware of any such findings in animal studies with PEGylated

proteins, nor were any signs of impaired immune function observed in the rat toxicity studies with pegvaliase.

In summary, although the daily dose of PEG delivered by pegvaliase appears to be higher than that of other approved therapeutic proteins containing PEG, the totality of the toxicology findings with pegvaliase, the known size-dependency of PEG tissue distribution (accumulation), and the experience with other PEGylated proteins suggests only a minimal concern for potential adverse effects from PEG accumulation during treatment with pegvaliase.

3. The Pharmacology/Toxicology review by Dr. Cai includes recommendations for labeling subsections 8.1 and 13.1, regarding the rat to human AUC margins from the combined fertility and embryo-fetal development study and the pre-/postnatal development study in rats. The recommended AUC margins in Dr. Cai's review were (b) (4) for the tested doses of 8 and 20 mg/kg/day, respectively, in both studies.

   In an amendment received on 05/07/2018, the Sponsor requested that these margins be changed to 4.2 and 19.4. The Sponsor explained that the rat $AUC_{0\text{-}72hr}$ values from the 26-week toxicity study were used to calculate the rat to human AUC ratios, and that the rat AUCs were generated from a dosing frequency of twice weekly. In contrast, the combined fertility and embryo-fetal development study and pre-/postnatal development study in rats utilized daily dosing. Therefore, the Sponsor multiplied the rat $AUC_{0\text{-}72hr}$ by 3, to generate values that are more appropriate for calculation of rat to human AUC multiples. The Sponsor's rationale is acceptable, and therefore we agree to the Sponsor's proposal to change the AUC margins to 4.2 and 19.4 for the doses of 8 and 20 mg/kg/day, respectively.

6 Clinical Pharmacology

6.1 Executive Summary

PALYNZIQ (pegvaliase) is a PEGylated recombinant *Anabaena variabilis* phenylalanine ammonia lyase (rAvPAL) that converts phenylalanine to ammonia and *trans*-cinnamic acid.

- **Proposed indication**: Pegvaliase is indicated to reduce blood phenylalanine concentrations in adult patients with Phenylketonuria who have uncontrolled blood phenylalanine concentrations greater than 600 micromol/L on existing management.
- **Proposed dosing regimen**: Pegvaliase is administered by SC injection. The Applicant proposed an Induction/Titration/Maintenance (I/T/M) dosage regimen. The proposed starting dosage is 2.5 mg once per week for 4 weeks. The pegvaliase dosage is titrated in a step-wise manner based on tolerability to achieve a maintenance dosage of 20 mg once daily or 40 mg once daily (the maximum dose). The pegvaliase dose is individualized based on patient tolerability, blood phenylalanine concentration and dietary protein and phenylalanine intake.
- **Proposed dosage forms**: 2.5 mg/0.5 mL, 10 mg/0.5 mL, and 20 mg/mL in a single-dose prefilled syringe. Note that each strength is expressed as the amount of rAvPAL in the solution.

The Applicant has evaluated the efficacy and safety of pegvaliase in PKU patients in two Phase 3 studies (165-301 and 165-302) and has additionally submitted results from five Phase 1 and Phase 2 studies (PAL-001, PAL-002, PAL-003, PAL-004, and 165-205) to support clinical pharmacology information of pegvaliase.

The key review findings are summarized in **Table 2**.

Table 2: Summary of Clinical Pharmacology Review Findings

Review Issues	Recommendations and Comments
Pivotal or supportive evidence of effectiveness	• The effectiveness of pegvaliase in reducing blood phenylalanine concentrations in adult PKU patients has been established in two Phase 3 trials. Refer to Section 7 of this multi-discipline review for more information. • The dose-response relationship for blood phenylalanine

	reduction provides supportive evidence of effectiveness. A trend towards higher blood phenylalanine reduction from baseline was observed in subjects receiving the 40 mg once daily (40 mg/day) dose compared to the 20 mg once daily (20 mg/day) dose.
General dosing instructions	• The pegvaliase dosing regimens and titration strategy based on patient tolerability and blood phenylalanine concentrations to achieve 20 mg/day and 40 mg/day maintenance regimens are appropriate. • The dose-response relationship for blood phenylalanine reduction and available clinical data support 40 mg/day as the maximum maintenance dosage. Discontinue pegvaliase in patients who have not achieved clinically meaningful reduction in blood phenylalanine concentration at the 40 mg/day dose. *See the approved full prescribing information for details of the recommended dosage regimens.*
Dosing in patient subgroups (intrinsic and extrinsic factors)	• The pegvaliase dose is individually titrated based on patient tolerability and blood phenylalanine concentrations. Further dose individualization based on intrinsic or extrinsic factors is not recommended.
Immunogenicity	• All patients had immunogenicity responses with formation of total anti-drug antibodies (total ADA, or TAb), anti-PAL IgM, anti-PAL IgG, anti-PEG IgM and/or anti-PEG IgG after treatment with pegvaliase. • Neutralizing antibodies (NAb) that are capable of inhibiting PAL enzyme activity were detected in majority of patients. • Development of ADAs was associated with reduced pegvaliase trough concentrations. • Development of ADAs was associated with reduced efficacy (i.e., decreased blood phenylalanine reduction). • Immunogenicity had impacts on safety. Development of ADAs, formation of circulating immune complexes (CICs), and decreased complement levels during induction and titration were associated with hypersensitivity adverse events (HAEs). Higher IgG-C3d CIC concentration changes from baseline were

	associated with a higher incidence of serious adverse events (SAEs), anaphylaxis, and study discontinuations due to AEs. The long-term impact of immunogenicity on safety is unknown. *See PMR recommendations.*
Drug interactions	• Majority of patients developed anti-PEG IgM and IgG antibodies after treatment with pegvaliase. We recommend monitoring of hypersensitivity reactions including anaphylaxis for patients receiving concomitant treatment with other PEGylated products.
Bridge between the to-be-marketed and clinical trial formulations	• The to-be-marketed prefilled syringe (PFS) formulation has been used in the Phase 3 clinical trials; therefore, there is no need to bridge the to-be-marketed formulation to the clinical trial formulation.

An OCP office level briefing was held on March 14, 2018.

6.1.1 Recommendations

From a clinical pharmacology standpoint, the BLA is acceptable to support approval of PALYNZIQ (pegvaliase) to reduce blood phenylalanine concentrations in adult patients with Phenylketonuria who have uncontrolled blood phenylalanine concentrations greater than 600 micromol/L on existing management.

6.1.2 Post-Marketing Requirements (PMRs)

The Clinical Pharmacology review team agrees with the Clinical review team that the Applicant conduct a prospective longitudinal observational study to collect long-term efficacy and safety data of pegvaliase. We have the following specific recommendation as part of the PMR:

- Conduct immunogenicity measurements including ADA, CIC, and complement levels at regular intervals and assess the impact of immunogenicity on safety.

The Clinical Pharmacology review team agrees with the OBP review team that the Applicant conduct the following PMRs:

- Revise the anti-PEG IgM anti-drug antibody assay in order to improve the drug tolerance and re-validate the assay.

- Revise the anti-PEG IgG anti-drug antibody assay in order to improve the drug tolerance and re-validate the assay.
- Evaluate the sensitivity of the anti-pegvaliase IgE ImmunoCAP assay to detect anti-PEG IgE antibodies, and to make modifications to the method as needed. Test samples from treated patients with the current or modified assay in the prospective study (according to PMR 1) who experience anaphylaxis episodes in order to more comprehensively examine the underlying mechanism of the anaphylaxis.

The Clinical Pharmacology review team recommends that the Applicant conduct the following PMRs:

- Re-evaluate anti-PEG IgM and IgG ADA in clinical samples from the phase 3 Trials 165-301 and 165-302 using the anti-PEG IgM and IgG ADA assays with improved drug tolerance. Re-assess the impact of anti-PEG IgM and IgG ADA on pharmacokinetics (PK), efficacy, and safety.
- Evaluate anti-PEG IgM and IgG ADA in samples from the observational study (according to PMR # 1) using the anti-PEG IgM and IgG ADA assays with improved drug tolerance. Assess the impact of anti-PEG IgM and IgG ADA on efficacy and safety.

6.2 Summary of Clinical Pharmacology Assessment

6.2.1 Pharmacology and Clinical Pharmacokinetics

- **Mechanism of Action:** Pegvaliase is a PEGylated phenylalanine ammonia lyase enzyme that converts phenylalanine to ammonia and *trans*-cinnamic acid. It substitutes for the deficient phenylalanine hydroxylase (PAH) enzyme activity and reduces blood phenylalanine concentrations in the body.

- **Pharmacodynamics:** Treatment of patients with pegvaliase resulted in the reduction of blood phenylalanine (Phe) concentrations. The reduction of blood phenylalanine concentrations diminished with decreased pegvaliase plasma concentrations.

- **Pharmacokinetics:** The PK profile of pegvaliase exhibits high between-subject and within-subject variability due to the heterogeneity of the immune response in patients with PKU. Higher antibody titers correlated with higher apparent clearance at steady state (CL_{ss}/F) and lower trough concentration of pegvaliase. In the first eight weeks during induction and titration, plasma pegvaliase concentrations were low to not measurable. At steady state during maintenance following administration of pegvaliase 20 mg/day and 40 mg/day dosage regimens, the mean

± SD (range) plasma trough pegvaliase concentrations were 11.2 ± 9.0 (0.21 to 29.6) mcg/mL and 10.4 ± 12.7 (0.18 to 43.1) mcg/mL, respectively.

- **Drug interactions:** Most patients treated with pegvaliase developed anti-PEG IgM and IgG antibodies. These anti-PEG antibodies may bind to PEG contained in other PEGylated products when co-administered with pegvaliase resulting in clinical consequences such as hypersensitivity. Therefore, we recommend monitoring of hypersensitivity reactions for patients receiving concomitant treatment of pegvaliase with other PEGylated products.

- **Immunogenicity:** All patients had immunogenicity responses with formation of total ADA, anti-PAL, and/or anti-PEG antibodies. Neutralizing antibodies were also detected in the majority of patients. Development of ADA was associated with reduced pegvaliase trough concentrations and reduced efficacy. Development of ADAs, formation of circulating immune complexes (CICs), and decreased complement levels during induction and titration were associated with hypersensitivity adverse events (HAEs). Higher IgG-C3d CIC concentration changes from baseline were associated with a higher incidence of serious adverse events (SAEs), anaphylaxis, and study discontinuation due to AEs.

6.2.2 General Dosing and Therapeutic Individualization

General Dosing

Overall, data from phase 3 Trials 165-301 and 165-302 support the proposed pegvaliase dosing regimens and titration strategy based on patient tolerability and blood phenylalanine concentrations to the achieve 20 mg/day and 40 mg/day maintenance regimens. The dose-response analysis results support 40 mg/day as the maximum maintenance dosage.

Therapeutic Individualization

Dose individualization based on intrinsic or extrinsic factors is not necessary for the following considerations: (1) the pegvaliase dose is individually titrated based on patient tolerability and blood Phe concentrations, (2) the PK profile of pegvaliase exhibits high between-subject and within-subject variability primarily due to the heterogeneity of the immune response in patients with PKU, and (3) the dose of pegvaliase may be affected or adjusted based dietary protein and/or Phe intake.

Outstanding Issues

There are no outstanding issues that would preclude the approval of pegvaliase from a Clinical Pharmacology's perspective.

An association between immunogenicity of pegvaliase and safety (e.g., hypersensitivity) has been shown in phase 3 trials; however, the limited available data in the clinical development program do not provide insights into the potential long-term impact of immunogenicity on safety, especially given the variations in the time course of the immunogenicity profiles that have not been adequately characterized in the current BLA. For example, mean CIC concentrations declined from peak values at Week 12 but did not return to baseline levels with the currently available data. In addition, mean C3 and C4 concentrations did not return to baseline levels and remained below the lower limit of normal in 40% and 14% of subjects, respectively, even after four years of pegvaliase treatment. These data suggest that a longer duration of study would be needed to further characterize the immunogenicity profiles of CIC, C3, and C4 concentrations and to assess their potential impacts on long-term safety.

The anti-PEG antibody assays have poor drug tolerance levels of 1 ng/mL and 10 ng/mL of pegvaliase for the detection of anti-PEG IgG and anti-PEG IgM, respectively. These drug tolerance levels are lower than the mean drug concentrations observed in the clinical studies indicating the limitation of these assays to detect ADA in the presence of pegvaliase. Because the mean drug concentrations during maintenance are expected to be higher than the drug tolerance levels of the assays, the observed declines in the immunogenicity incidences and mean antibody titers of anti-PEG antibodies cannot be ascertained, and consequently, the impacts of anti-PEG antibodies on PK, efficacy, and safety remain to be determined with data from improved assays.

We recommend the use of product labeling to communicate the immunogenicity findings in the BLA. We also recommend PMR studies to address the outstanding issues discussed above. See section 6.1.2 for details of the PMR recommendations.

6.3 Comprehensive Clinical Pharmacology Review

6.3.1 General Pharmacology and Pharmacokinetic Characteristics

A summary of the general clinical pharmacology, PK, and immunogenicity of pegvaliase is provided in **Table 3**.

Table 3: Summary of Clinical Pharmacology, Pharmacokinetics, and Immunogenicity of pegvaliase.

Pharmacology	
Mechanism of action	PKU is caused by the deficiency of phenylalanine hydroxylase (PAH). Pegvaliase is a PEGylated phenylalanine ammonia lyase enzyme that converts phenylalanine to ammonia and *trans*-cinnamic acid. It substitutes for the deficient PAH enzyme activity and reduces blood phenylalanine concentrations in the body.
Pharmacodynamics	Treatment of patients with pegvaliase resulted in the reduction of blood phenylalanine (Phe) concentrations. The reduction of blood Phe concentrations diminished with decreased pegvaliase plasma concentrations. In clinical Trial 165-301, the mean (SD) blood Phe concentrations at baseline were 1241 (390) µmol/L and 1224 (384) µmol/L in the 20 mg/kg and 40 mg/day dose groups, respectively. In the first eight weeks during induction and titration, there were minimal reductions in blood Phe concentrations and pegvaliase concentrations were not detectable in majority of subjects. After Week 8, as subjects were titrated to higher doses and mean trough pegvaliase concentrations increased, the mean Phe concentrations were reduced in both dose groups, with greater Phe reductions in the 40 mg/day dose group than the 20 mg/day dose group. The mean (SD) blood Phe reductions were 404 (505) µmol/L and 563 (621) µmol/L at Week 28, and 356 (540) µmol/L and 525 (679) µmol/L at Week 36 in the 20 mg/day and 40 mg/day dose groups, respectively.
General information	
Bioanalysis of PK samples	PK samples were analyzed by an enzyme-linked immunosorbent assay (ELISA) that used rabbit monoclonal anti-PEG IgG as the capture reagent and rabbit polyclonal anti-rAvPAL IgG as detection reagent. Acid pre-treatment of samples prior to analysis dissociates the ADA-bound pegvaliase complexes resulting in the measurement of total pegvaliase concentrations.
PK variability	The PK profile of pegvaliase exhibits high inter-subject and intra-subject variability due to the heterogeneity of the immune response in patients with PKU. Higher antibody titers correlated with higher CL_{ss}/F and lower pegvaliase trough concentrations.

Drug concentrations at steady state	In the first eight weeks during induction and titration, plasma pegvaliase concentrations were low to not measurable. At steady state during maintenance following administration of pegvaliase 20 mg/day and 40 mg/day, the mean ± SD (range) plasma trough pegvaliase concentrations were 11.2 ± 9.0 (0.21 to 29.6) mcg/mL and 10.4 ± 12.7 (0.18 to 43.1) mcg/mL, respectively.
Dose linearity	Due to the impact of immunogenicity on PK, dose linearity was not observed for the doses of 20 mg/day and 40 mg/day evaluated in the Phase 3 studies.
ADME The following pharmacokinetic parameters were observed in patients with PKU following multiple SC administration of pegvaliase 20 mg and 40 mg once daily, unless otherwise specified.	
Absorption	The median T_{max} was approximately 8 hours. The mean ± SD (range) $C_{max,ss}$ was 14.0 ± 16.3 (0.26 to 68.5) mcg/mL and 16.7 ± 19.5 (0.24 to 63.8) mcg/mL, respectively.
Distribution	The mean ± SD (range) Vz/F was 26.4 ± 64.8 (1.8 to 241) L and 22.2 ± 19.7 (3.1 to 49.4) L, respectively.
Elimination	The mean ± SD (range) CL_{ss}/F was 0.39 ± 0.87 (0.018 to 3.66) L/h and 1.25 ± 2.46 L/h (0.034 to 8.88), respectively. The mean ± SD (range) $t_{1/2}$ was 47 ± 42 (14 to 132) hours and 60 ± 45 (14 to 127) hours, respectively.
--Metabolism	The metabolism of phenylalanine ammonia lyase is expected to occur via catabolic pathways and be degraded into small peptides and amino acids.
--Excretion	The route of elimination of pegvaliase has not been studied in human subjects.
Immunogenicity	
Antidrug antibodies (ADAs) and immunogenicity assays	Subjects treated with pegvaliase were assessed for antibodies against the PAL protein (anti-PAL IgG and IgM), PEG moiety (anti-PEG IgG and IgM), total ADA (TAb), neutralizing antibodies (NAb) capable of inhibiting PAL enzyme activity, and anti-pegvaliase IgG 4 antibodies (IgG4). See Office of Clinical Pharmacology Appendices and OBP review for details of the immunogenicity assays.
ADA incidences and titers	A summary of ADA incidences and titers in Phase 3 studies are presented in **Table 4**.

	Pre-existing antibodies were detected in 6.6% (anti-PAL IgG), 50% (anti-PAL IgM), 53% (anti-PEG IgG), 45% (anti-PEG IgM), 31% (TAb), and 13% (IgG4) of subjects at baseline. For the I/T/M Population, all patients treated with pegvaliase developed a sustained total TAb response with majority of patients (91%; N = 235/258) becoming positive by Week 4 of treatment. Mean TAb titers peaked 2 weeks after pegvaliase initiation and were sustained throughout treatment (greater than 1 year after treatment initiation). Anti-PAL IgM antibodies were detected in all patients with majority of treated patients (98%; N = 265/270) becoming positive for anti-PAL IgM by 2 months after treatment initiation. Anti-PAL IgG antibodies were detected in almost all patients (N = 226/227) by 4 months after treatment initiation. Mean PAL IgM and IgG titers peaked at approximately 3 and 6 months, respectively, after treatment initiation and remained elevated throughout treatment (greater than 1 year after treatment initiation). Drug -induced anti-PEG IgM and IgG antibodies were detected in majority of patients (98%; N = 277/284 for IgM; and 278/284 for IgG) with mean titers peaking at 1 to 3 months after treatment initiation. The incidence and mean titer values of anti-PEG antibodies decreased over time; however, these decreases are likely attributable to the low drug tolerance of the anti-PEG antibody assays and relatively higher drug concentrations in the maintenance Phase of treatment. NAb antibodies were detected on at least one measurement in majority of patients (88%; N = 249/284) over time. Mean NAb titers plateaued at 16 to 20 weeks and remained present throughout treatment (greater than 1 year after treatment initiation). Sixty eight of 285 patients were tested for both anti-PAL IgE antibodies and anti-pegvaliase IgE antibodies during routine study visits (not at times of anaphylaxis episodes) or during additional hypersensitivity visits for hypersensitivity reactions. Of those 68 patients, 5 (7%) tested positive at least once for anti-PAL IgE antibodies but none tested positive for anti-pegvaliase IgE antibodies.
CIC and C3, C4	Overall, mean IgG CIC and IgM CIC concentrations increased and were highest at Week 12 during early treatment. These concentrations

	returned towards but not back to baseline with longer-term treatment with the currently available data. In parallel with these CIC changes, mean complement C3 and C4 concentrations declined from baseline to reach nadirs at 3 to 6 months and then slowly increased towards baseline levels. However, mean baseline C3/C4 concentrations were not restored. IgG C3d-CIC and IgM C3d-CIC levels were above the upper limit of normal (ULN) in approximately 60% (N = 164/259) and 40% (106/259) of subjects, respectively, at Week 12 of pegvaliase treatment. The incidence of CIC values > ULN decreased over time, with high IgG C3d-CIC in 2 – 7% of subjects and high IgM-C3d CIC in 5 – 17% of subjects two to four years after pegvaliase administration. Approximately 61% of subjects (N = 110/180) had Complement C3 concentrations < lower limit of normal (LLN) at 6 months after treatment initiation and 38% of subjects (N = 94/248) had complement C4 concentrations < LLN at 3 months after treatment initiation. The incidence of low complement C3 and C4 concentrations declined over time, but approximately 39% (N = 19/49) and 12% (N = 6/49) of subjects had low C3 and C4 concentrations, respectively, at 36 months after treatment initiation. Approximately 33 % (N = 4/12) and 8 % (N = 1/12) of subjects had low C3 and C4 concentrations at 45 months after treatment initiation.
Impact on PK	Development of ADAs was associated with increased pegvaliase clearance and reduced pegvaliase trough concentrations.
Impact on efficacy	Development of ADAs was associated with reduced efficacy (i.e., decreased blood phenylalanine reduction). Subjects with higher antibody titers appeared to be associated with diminished blood Phe reductions.
Impact on safety	An association between antibody response and adverse events was observed in pegvaliase clinical studies. The highest frequency of hypersensitivity AEs (HAEs) reactions occurred within the first 6 months of pegvaliase treatment when the mean CIC concentrations were at the highest and mean C3 and C4 complement concentrations were at the lowest. Mean CIC concentrations decreased and complement levels increased over time as the exposure-adjusted rate of hypersensitivity reaction decreased. The paralleled time course of these immune-related analytes and HAEs during early treatment suggests that the predominant mechanism of hypersensitivity reactions was Type III immune complex-

	medicated hypersensitivity. During late treatment (one year after pegvaliase initiation), higher antibody titers appeared to be associated with a higher incidence of HAEs. Higher IgG-C3d CIC changes from baseline were associated with a higher incidence of SAEs, anaphylaxis, and study discontinuations due to AEs. Approximately 41% of 129 Phase 3 subjects who initially enrolled in Study 165-301 had IgG CIC concentration changes higher than the median value discontinued pegvaliase; 14.7% of these subjects discontinued the study due to adverse events. In contrast, among 130 subjects with CIC concentration changes below the median value, 16.9% discontinued from study and 2.3% discontinued the study due to adverse events. Twenty-five of 26 subjects who had anaphylaxis were tested for anti-pegvaliase IgE antibodies; one subject was not tested. Of the 25 subjects tested for anti-pegvaliase IgE antibodies, 24 subjects were tested negative. One subject screened positive for anti-pegvaliase IgE antibodies but had insufficient sample to confirm IgE positivity. This patient tested negative for anti-pegvaliase IgE at routine visits prior to and after the anaphylaxis episode (not at times of anaphylaxis).

Table 4: Incidences and Titers of Antidrug Antibodies in Phase 3 Studies

	Incidence			Mean Titer [Week] (Min, Max)		
	Baseline	**Peak in 165-301 [Week]**	**Overall in 165-302**	**Baseline**	**Peak in 165-301**	**Overall in 165-302**
Anti-PAL IgG	6.6	100 [W16]	100	59 (0, 12150)	1258270 [W36] (450, 8857350)	1109811 (641, 6889050)
Anti-PAL IgM	50.2	98.5 [W16]	99.1	244 (0, 8201)	3315 [W12] (115, 12900)	1700 (27, 83607)
Anti-PEG IgG	52.5	96.4 [W8]	50.7	36 (0, 810)	7942 [W3] (0, 196830)	40.4 (0, 2970)
Anti-PEG IgM	44.8	96.0 [W8]	90.7	178 (0, 2404)	47618 [W12] (0, 5360000)	1061 (0, 90700)

TAb	30.9	100 [W24]	100	206 (0, 10700)	67137 [W3] (0, 1060000)	16291 (153, 100557)
NAb	0.4	77.1 [W36]	95.8	0 (0, 18)	590 [W32] (0, 13122)	287 (0, 3402)
IgG4	12.6	99.4 [W72]	98.2	8 (0, 435)	8921587 [W96] (0, 83900000)	-

6.3.2 Clinical Pharmacology Questions

6.3.2.1 Does the clinical pharmacology program provide supportive evidence of effectiveness?

The effectiveness of pegvaliase in reducing blood Phe concentrations in adult PKU patients has been established in two phase 3 trials. See sections 7 and 8 of this multi-discipline review for evaluation and details of the study design and results of the phase 3 trials.

In the first eight weeks during induction and titration, there were minimal reductions in blood Phe concentrations and pegvaliase concentrations were not detectable in majority of subjects. After Week 8, as subjects were titrated to higher doses and mean trough pegvaliase concentrations increased, the reduction of Phe concentrations was observed, indicating the reduction of blood Phe concentrations was dependent on plasma pegvaliase concentrations although an exact exposure-response relationship could not be characterized due to limitations of the PK assay and variability of the PK data.

During maintenance, a trend towards higher blood Phe reduction from baseline was observed in the 40 mg/day dosage compared to the 20 mg/day dosage. The dose-response relationship for blood Phe reduction provides supportive evidence of effectiveness. See section 6.3.2.2 below for more information.

6.3.2.2 Is the proposed dosing regimen appropriate for the general patient population for which the indication is being sought?

Yes, the proposed dosing regimens, starting with a low dose and then titrating upwards based on patient tolerability and blood Phe concentration with an aim to reach the lowest effective maintenance dose of 20 mg/day or 40 mg/day, are appropriate for the treatment of patients with PKU.

Overall, the results of the Phase 3 studies 165-301 and 165-302 have supported the following dosing regimen recommendations for pegvaliase:

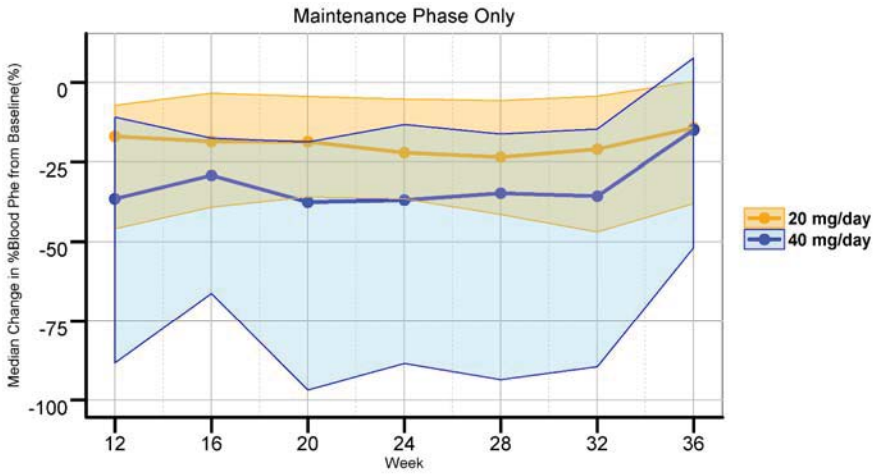
- During the induction and titration phase, increase the pegvaliase dose in a step-wise manner based on patient tolerability to achieve a dosage of 20 mg/day.
- During the maintenance phase, further increase in the pegvaliase dose from 20 mg/day to 40 mg/day may be necessary to achieve the desired reduction in blood Phe concentrations.
- In the event of inadequate Phe reduction after 16 weeks of pegvaliase treatment with the maximum dosage of 40 mg/day during maintenance, discontinuation of pegvaliase treatment is appropriate.
- Adjust dietary protein and Phe intake based on individual blood Phe concentrations.

Clinical Pharmacology's assessment that supports the dose escalation from 20 mg/day to 40 mg/day during maintenance is provided below.

Dose-response (20 mg/day versus 40 mg/day) for blood Phe reduction in Trial 165-301:
Although Trial 165-301 was not designed as an efficacy trial, it is possible to evaluate dose-response relationships in subjects receiving a fixed maintenance dose of 20 mg/day or 40 mg/day. Overall, the dose-response relationship suggests a trend of higher blood Phe reduction in the 40 mg/day group compared to the 20 mg/day group, as summarized below:

- Comparison of the percent change of blood Phe concentration from baseline in patients who reached the target maintenance dose showed that both the median and first quartile percent reduction in blood Phe concentration were higher in the 40 mg/day group compared to the 20 mg/day group (**Figure 1**).
- The mixed-effect repeated measures analyses showed a trend of higher blood Phe reduction in the 40 mg/day group after adjusting for significant covariates (e.g., blood Phe concentration and protein intake from intact food at baseline). The least squares mean of the difference between the 20 mg/day group and the 40 mg/day group based on the population of subjects who entered maintenance was 17.77% (95% CI: 7.23%, 28.3%).
- See OCP Appendices for additional information and details of the analysis.

Figure 1: %Change in Blood Phe Concentration from Baseline by Dose Group in Patients Who Reached the Planned Maintenance Dose in Study 165-301



Treatment	Week 12	Week 16	Week 20	Week 24	Week 28	Week 32	Week 36
20 mg/day	89	102	100	102	102	99	38
40 mg/day	67	78	83	81	80	78	28

Solid colored line: Median. Shaded Area: 1st and 3rd quartile. Table: Number of subjects by dose group at each assessment timepoint. The ITT population consisted of 261 subjects: 131 subjects from the 20 mg/day group and 130 subjects from 40 mg/day group. There were no meaningful differences in demographics or other baseline characteristics between the two randomized dose groups. *(Source of data: FDA Reviewer's analysis based on dataset "adeff.xpt" and "adex.xpt" in study 165-301)*

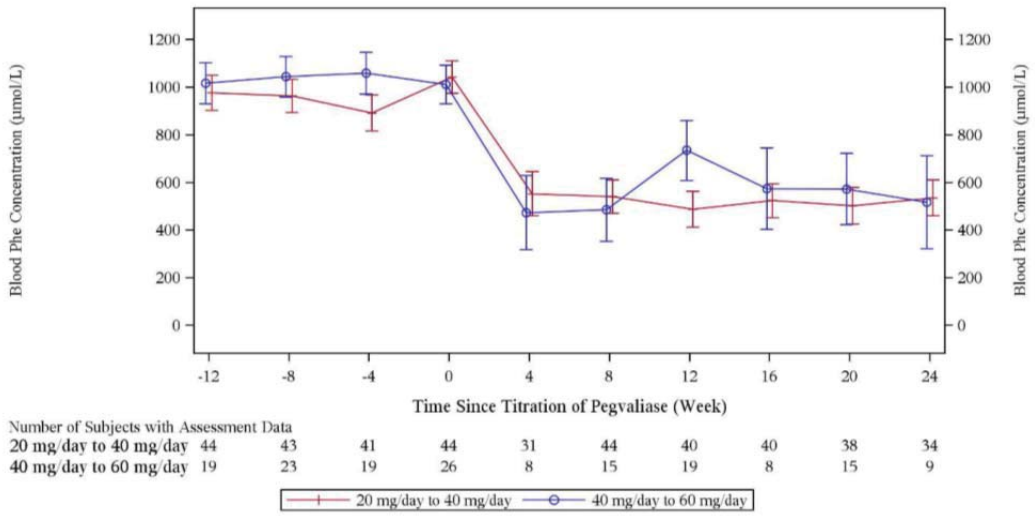
Effect of dose escalation on blood Phe reduction in Trial 165-302 (Part 4)

The effect of pegvaliase dose escalation on blood Phe reduction is demonstrated by the subgroup analysis results in Part 4 of Study 165-302. The subgroup analysis compared blood Phe concentrations before and after dose escalation in 63 subjects. Of the 63 subjects, 44 subjects received the pegvaliase dose of 20 mg/day for ≥ 12 weeks before dose escalation and 19 subjects received the pegvaliase dose of 40 mg/day for ≥ 12 weeks before dose escalation. The highest pegvaliase dose reached after dose escalation in these subjects is summarized in **Table 5**. The results show the following:

- Dose escalation (from 20 mg/day to 40 mg/day or from 40 mg/day to 60 mg/day) resulted in reduction of mean blood Phe concentrations (**Figure 2**). The mean ± SD blood Phe concentration decreased from 1042 ± 454 micromol/L at the time prior to dose escalation (i.e., Week 0) to 552 ± 516 micromol/L at Week 4 after dose escalation in subjects who had dose escalation from 20 mg/day to 40 mg/day. Similarly, the mean ± SD blood Phe concentration declined from 1011 ± 418 micromol/L at Week 0 to 473 ± 439 micromol/L at Week 4 in subjects who had dose escalation from 40 mg/day to 60 mg/day.

- Of the 44 subjects who were on a pegvaliase dose of 20 mg/day before dose escalation, 22 (50%) of subjects achieved a sustained Phe response ≤ 600 micromol/L when pegvaliase dose was escalated to 40 mg/day (**Table 5**).
- Of the 19 subjects who were on a pegvaliase dose of 40 mg/day before dose escalation, 5 (26.3%) of subjects achieved a sustained Phe response ≤ 600 micromol/L when pegvaliase dose was escalated to 60 mg/day (**Table 5**).

Figure 2: Mean Blood Phe Concentrations Before and After Dose Escalation in Part 4 of Trial 165-302



(Source of data: Figure 3.1 of Response to Clinical Pharmacology Information Request dated 19January2018; SDN 47)

Table 5: Summary of Blood Phe Response in Subjects Who Received Dose Escalation in Part 4 of Trial 165-302

Pegvaliase Dose Prior to Escalation	Highest Pegvaliase Dose Reached after Dose Escalation	Sustained Blood Phe ≤ 600 micromol/L[#]	Sustained Blood Phe ≤ 600 micromol/L Not Achieved
20 mg/day (N = 44)	40 mg/day (N = 32)	**22 (50.0%)**	10 (22.7%)
	60 - 120 mg/day (N = 12)	7 (15.9%)	5 (11.4%)
40 mg/day (N = 19)	60 mg/day (N = 17)	**5 (26.3%)**	12 (63.1%)

	80 - 120 mg/day (N = 2)	2 (10.6%)	-

Subjects met the following criteria were included in the subgroup analysis: (1) Subjects received at least 12 weeks of dose on 20 mg/day with at least 80% compliance prior to increasing to 40 mg/day, (2) Subjects had at least 12 weeks on 40 mg/day with 80% compliance after the dose increase, and (3) Subjects were excluded from the analysis if they had missing TAb data at the time of dose titration or if they received placebo in Part 2 within 12 weeks prior to dose increase. Similar criteria were followed for subjects who titrated from 40 mg/day to 60 mg/day. Of the 44 subjects received 20 mg/day prior to dose escalation, 32 and 12 subjects were dose titrated to 40 mg/day and 60 - 120 mg/day, respectively. Of the 19 subjects received 40 mg/day prior to dose escalation, 17 and 2 subjects were dose adjusted to 60 mg/day and 80 - 120 mg/day, respectively.

[#]Blood Phe concentration ≤ 600 micromol/L for three consecutive measurements of at least 16 weeks apart with no obvious trend of blood Phe concentration increase and no more than two Phe concentration measurements > 600 micromol/L. *(Source of data: Reviewer's analysis based on dataset "adcic.xpt" of Response to Clinical Pharmacology Information Request dated 19January2018; SDN 47)*

Dose-response for safety before and after dose escalation

Based on the limited number of subjects, comparison of the adverse event rates for HAEs, injection site reactions (ISRs), and arthralgia before and after dose escalation did not show an apparent dose-response relationship between 20 mg/day and 40 mg/day (**Table 6**) or between 40 mg/day and 60 mg/day (**Table 7**).

Table 6: Adverse Events for Subjects on 20 mg/day Dose prior to Titration and on 40 mg/day Dose after Titration

	Before Dose Titration N=44		After Dose Titration N=44	
	Subjects n (%) 95% CI (%)	Incidence Rate[a] (95% CI) Events (n) Event Rate[b] (95% CI)	Subjects n (%) 95% CI	Incidence Rate[a] (95% CI) Events (n) Event Rate[b] (95% CI)
Exposure (weeks)		23.5		40.8
HAEs	27 (61) 45, 76	1.15 (0.79, 1.68) 181 7.71 (6.66, 8.92)	26 (59) 43, 74	0.64 (0.43, 0.94) 264 6.46 (5.73, 7.29)
ISRs[c]	26 (59) 43, 74	1.11 (0.75, 1.63) 359 15.29 (13.79, 16.95)	28 (64) 48, 78	0.69 (0.47, 0.99) 128 3.13 (2.64, 3.73)
Arthralgia[d]	18 (41) 26, 57	0.77 (0.48, 1.22) 89 3.79 (3.08, 4.67)	20 (45) 30, 61	0.49 (0.32, 0.76) 42 1.03 (0.76, 1.39)

HAEs, hypersensitivity reactions; ISRs, injection site reactions; CI, confidence interval *(Source of data: Table 2 of Response to Clinical Pharmacology Information Request dated 16February2018; SDN 51)*

Table 7: Adverse Events for Subjects on 40 mg/day Dose prior to Titration and on 60 mg/day Dose after Titration

	Before Dose Titration N=26		After Dose Titration N=26	
	Subjects n (%) 95% CI (%)	Incidence Rate[a] (95% CI) Events (n) Event Rate[b] (95% CI)	Subjects n (%) 95% CI	Incidence Rate[a] (95% CI) Events (n) Event Rate[b] (95% CI)
Exposure (weeks)		14.7		22.4
HAEs	17 (65) 44, 83	1.16 (0.72, 1.86) 49 3.33 (2.52, 4.41)	16 (62) 41, 80	0.71 (0.44, 1.17) 52 2.32 (1.77, 3.05)
ISRs[c]	14 (54) 33, 73	0.95 (0.56, 1.61) 85 5.78 (4.68, 7.15)	13 (50) 30, 70	0.58 (0.34, 1.00) 53 2.37 (1.81, 3.10)
Arthralgia[d]	12 (46) 27, 67	0.82 (0.46, 1.44) 27 1.84 (1.26, 2.68)	8 (31) 14, 52	0.36 (0.18, 0.71) 18 0.80 (0.51, 1.28)

HAEs, hypersensitivity reactions; ISRs, injection site reactions; CI, confidence interval. *(Source of data: Table 2 of Response to Clinical Pharmacology Information Request dated 16 February 2018; SDN 51)*

6.3.2.3 Is an alternative dosing regimen or management strategy required for subpopulations based on intrinsic factors?

No, an alternative dosing regimen is not required for subpopulations based on intrinsic factors, for the following consideration:

- Pegvaliase dosing is individually titrated based on patient tolerability and blood Phe concentrations.
- The PK profile of pegvaliase exhibits high between-subject and within-subject variability due to the heterogeneity of the immune response in patients with PKU. In pegvaliase clinical trials, all patients had immunogenicity responses with formation of total, anti-PAL, and/or anti-PEG antibodies. Anti-drug antibodies were found to affect both the PK and efficacy of pegvaliase. The currently available clinical data do not support dose adjustment based on the subject immunogenicity status.
- In practice, the optimal dose of pegvaliase may be affected by or adjusted based on dietary protein and/or Phe intake.

6.3.2.4 Are there clinically relevant food-drug or drug-drug interactions, and what is the appropriate management strategy?

Pegvaliase is a rAvPAL conjugated with linear 20 kDa N-hydroxysuccinimide (NHS)-methoxypolyethylene glycol (NHS-PEG). The metabolism of rAvPAL is expected to occur via catabolic pathways and be degraded into small peptides and amino acids. To our knowledge, metabolism through cytochrome P450 (CYP) enzymes does not represent a

considerable pathway for PEG elimination, although the exact route of elimination of NHS-PEG has not been studied. Therefore, direct drug-drug interactions between pegvaliase and small molecule drugs that are metabolized by cytochrome P450 (CYP) enzymes are unlikely.

Food-drug interactions are not applicable as pegvaliase is administered by SC injection.

Effect of anti-PEG antibodies on other PEGylated products
Most patients treated with pegvaliase develop anti-PEG IgM and IgG antibodies. These anti-PEG antibodies may bind to PEG contained in other PEGylated products when co-administered with pegvaliase resulting in clinical consequences such as hypersensitivity and/or anaphylaxis.

In the phase 1 single dose study of pegvaliase in PKU patients (Study PAL-001), two patients receiving concomitant injections of medroxyprogesterone acetate injectable suspension, a formulation containing PEG 3350, experienced hypersensitivity reactions. One of the two patients experienced anaphylaxis on Day 40 after a single pegvaliase dose of 0.001 mg/kg within 10 minutes following medroxyprogesterone acetate injection suspension. The other subject experienced a hypersensitivity reaction on Day 15 after a single pegvaliase dose of 0.01 mg/kg within 15 minutes following medroxyprogesterone acetate injection suspension, and subsequently experienced anaphylaxis on Day 89 within 30 minutes after the next dose of medroxyprogesterone acetate injectable suspension. Both patients had high anti-PEG IgG antibody titers at or around the time of the reactions (**Table 8**).

Because of the potential risk for cross reactivity of anti-PEG antibodies and the observed clinical data, we recommend monitoring of hypersensitivity reactions including anaphylaxis for patients receiving concomitant treatment of pegvaliase with other PEGylated products.

Table 8: Summary of Hypersensitivity and Anaphylaxis Events and anti-PEG Antibody Titers for the two Subjects Receiving Concomitant Treatment of Pegvaliase with Medroxyprogesterone Acetate Injectable Suspension in Study PAL-001

	Subject (b) (6)	**Subject** (b) (6)
Pegvaliase dose	0.001 mg/kg (single dose)	0.01 mg/kg (single dose)
Reaction (Day after pegvaliase injection)	Hypersensitivity SAE (Day 40)	Non-serious HAE (Day 15) Urticarial/anaphylactic reaction SAE (Day 89)
Prior medroxyprogesterone use	Had been receiving medroxyprogesterone acetate injections for the "last 6 years."	No prior history of urticaria and had tolerated previous Depo-Provera injections well.
Anti-PEG IgG/IgM Titers		
Baseline	< 50/negative	< 50/negative
Day 15	4050/positive	36450/positive
Day 29	4050/positive	12150/positive
Day 43	12150/positive	12150/positive

(Source of data: Sections 12.3.1.2 and 14.3.3.1 and Listing 16.2.8.7 of PAL-001 CSR)

7 Sources of Clinical Data and Review Strategy

7.1 Table of Clinical Studies

Table 9: Summary of Key Phase 2 and 3 Clinical Trials for the I/T/M Population

Trial	Design	Regimen/ schedule/ route	Endpoints	Duration	No. of patients	Population	No. of Centers and Countries
Phase 3							
165-301	Open-label, randomized to 2 doses (low or high maintenance dose), safety and tolerability study	Induction Period: fixed-dose 2.5 mg once weekly for 4 weeks Titration Period: increase weekly dose to a daily treatment regimen of 20 mg once daily or 40 mg once daily (Weeks 5 up to 34) Maintenance Period: 20 mg once daily or 40 mg once daily for an additional 2 weeks up to Week 36; SC injection; VS[27] or PFS[28] for self-administration	Primary endpoint was safety, secondary endpoint was change from baseline in blood Phe concentration by visit week and randomized dose group, tertiary endpoints were attention and mood tests, protein intake measurements	Up to 36 weeks	261; 203 out of 261 subjects transitioned to Study 165-302	18[29] to 70 years of age; patients with PKU naïve to pegvaliase.	31 study centers in the US
165-302	4-Part	Part 1 (Open-label Blood Phe Assessment): pegvaliase 20 mg once daily or 40 mg once daily	Primary endpoint was the change in blood Phe concentration	Part 1: up to 13 weeks	215	18[31] to 70 years of age; patients with PKU who previously received pegvaliase	29 study centers in the US

[27] Vial and syringe (VS)
[28] Prefilled syringe (PFS)
[29] The study age criterion was modified with a protocol amendment to increase the lower age limit from 16 years old to 18 years old (FDA Advice/information request; 14MAY2014).

Trial	Design	Regimen/ schedule/ route	Endpoints	Duration	No. of patients	Population	No. of Centers and Countries
		(via VS) until a mean blood Phe reduction of ≥20% from baseline levels is achieved to go into Part 2.[30] <u>Part 2 (Randomized Withdrawal, Double-blind):</u> pegvaliase 20 mg once daily or 40 mg once daily or matching placebo (via VS) <u>Part 3a and 3b (PK/PD Assessments):</u> pegvaliase administered using VS for 1 week in Part 3a, and 4 weeks using PFS in Part 3b, followed by a 1-week treatment break. <u>Part 4 (Open-label, Long-Term Extension):</u> 40 mg once daily	from baseline of Part 2 to Week 8 of Part 2, secondary endpoints were changes from the baseline of Part 2 to Week 8 of Part 2 for scores of inattention and mood (ADHD-RS, POMS scales)	Part 2: 8 weeks Part 3: 6 weeks Part 4: Up to 212 weeks			

[31] The study age criterion was modified with a protocol amendment to increase the lower age limit from 16 years old to 18 years old (FDA Advice/information request; 14MAY2014).
[30] Subjects who were unable to achieve the ≥ 20% blood Phe reduction after 13 weeks in Part 1 or were unable to maintain the 20 mg/day or 40 mg/day dose in Part 1 due to AEs transitioned directly to study 165-301 Part 4 and did not participate in Part 2 or 3 of the study.

Trial	Design	Regimen/ schedule/ route	Endpoints	Duration	No. of patients	Population	No. of Centers and Countries
		using PFS. Subjects allowed to titrate up to 60mg once daily per investigator discretion.					
165-303	Substudy to evaluate executive function (uncontrolled)	No study drug administered	Evaluate executive function as measured by Cambridge Neuropsychological Test Automated Battery (CANTAB)	Up to 63 weeks	9	18 to 70 years of age; patients with PKU concurrently treated with pegvaliase or placebo in Study 165-302	4 study centers in the US
Phase 2							
PAL-003	Open-label, long-term extension	0.001 to 1.0 mg/kg per individual injection, or a fixed weekly dose ranges from 2.5 mg to a maximum of 375 mg, each subject's dose is adjusted as needed to attempt to attain or maintain blood Phe concentrations of 60 to 600 µmol/L; SC injection using VS or PFS	Blood Phe concentration, secondary objectives of safety and tolerability, immunogenicity	Up to 86 months or until study is terminated	68	Patients with PKU who completed PAL-002, PAL-004, or 165-205	14 study centers in the US
165-205	Open-label, dose-finding study to evaluate multiple	Induction: 4-8 week induction at 2.5 mg/week fixed dose,	Blood Phe concentration, secondary	24 weeks	24	16 to 70 years of age; patients with PKU	6 study centers in the US

Trial	Design	Regimen/ schedule/ route	Endpoints	Duration	No. of patients	Population	No. of Centers and Countries
	schedules involving induction, titration, and maintenance dosing	followed by titration for a minimum of 4 weeks until blood Phe reduction to ≤ 600 µmol/L and maintained reduction without dose modification for ≥ 4 weeks (to maximum weekly dose of 75 mg/day [375 mg/week])	objectives immunogenicity, PK profile				

Source: Table 5.2: Listing of Clinical Studies in the BLA submission, dated 6/30/2017.

7.2 Review Strategy

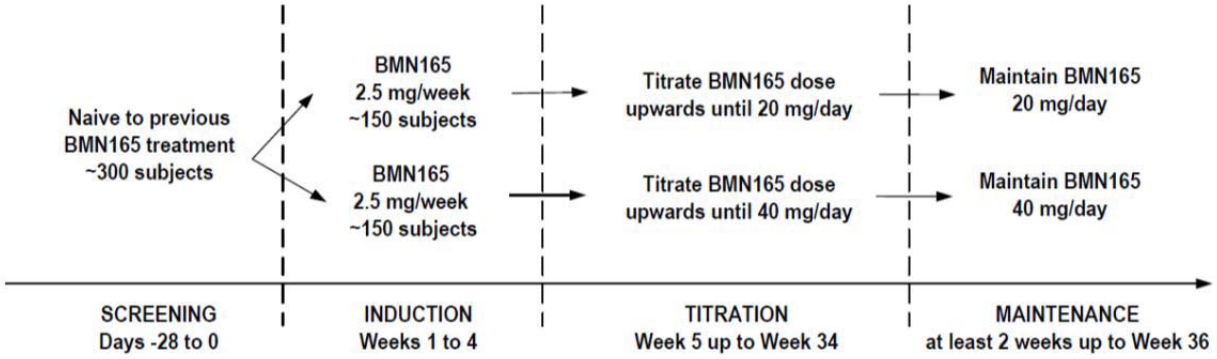
The sources of data used for the evaluation of the efficacy and safety of pegvaliase for the proposed indication primarily included the pivotal phase 3 Trials 165-301 and 165-302. The focus of the efficacy review was on Trial 165-302 Part 2, which was the only placebo-controlled period. The focus of the safety review was on the Induction/Titration/Maintenance population from the parent Trials 165-205 and 165-301 and their feeder Trials PAL-003 and 165-302. This population is most relevant to use after approval because it includes all subjects given the I/T/M dosing regimen as in the proposed label and will inform prescribers and patients with postmarketing use. The details of the safety review approach are discussed below in section 8.2.1. The efficacy and safety sections below represent an integrated approach by the clinical, statistical, and clinical pharmacology team members. This multi-disciplinary assessment enables an integrated analysis of the clinical data incorporating different perspectives, and strengthens the review team's final conclusions on the safety and efficacy of the product in the intended patient population.

8 Statistical and Clinical Evaluation

8.1 Review of Relevant Individual Trials Used to Support Efficacy

8.1.1 Trial 165-301: "A Phase 3, Open-Label, Randomized, Multi-Center Study to Assess the Safety and Tolerability of an Induction, Titration, and Maintenance Dose Regimen of BMN 165 Self-Administered by Adults with Phenylketonuria Not Previously Treated with BMN 165"

Figure 3: Overview of 165-301 Trial Design



Source: Applicant's submission, dated 6/30/2017, Module 5.3.5.2 165-301 Protocol and Protocol Amendments, page 9.

Objectives

- Primary: To characterize the safety and tolerability of pegvaliase during induction, titration, and maintenance dosing up to 20 mg/day or 40 mg/day in treatment naïve subjects with PKU.
- Secondary: To evaluate blood Phe concentration after treatment.
- Tertiary:
  - To characterize the dietary protein intake from medical and intact foods.
  - To characterize and evaluate change in inattention, hyperactivity, and

mood symptoms.

- To evaluate trough plasma concentrations of pegvaliase.

Trial Centers and Number of Subjects

261 subjects enrolled in 31 study centers in the United States.

Trial Population

Key Inclusion Criteria

- Diagnosis of PKU with blood Phe concentration >600 µmol/L at screening and average blood Phe concentration of >600 µmol/L over the past 6 months (per available data)
- No previous exposure to BMN 165.
- ≥18 years and ≤70 years of age at screening (subjects <18 years who were already enrolled were allowed to continue trial participation)
- If taking Kuvan, have a treatment end date ≥14 days prior to first dose
- Have identified a competent person or persons who are ≥ 18 years of age who can observe the subject during study drug administration and for a minimum of 1 hour following administration until dose titration has completed and if needed upon return to dosing after an AE and per investigator determination (a home healthcare nurse may perform the study drug observation).
- Have documented approval from a study dietitian confirming that the subject can maintain their diet in accordance with Section 9.6 (see protocol and diet section below).
- If applicable, maintained stable dose of medication for ADHD, depression, anxiety, or other psychiatric disorder for ≥8 weeks prior to enrollment and willing to maintain stable dose throughout study unless a change is medically indicated.

Key Exclusion Criteria

- Use of any medication intended to treat PKU (except Kuvan), including the use of large neutral amino acids, within 2 days prior to first dose.
- Use or planned use of any injectable drugs containing PEG other than pegvaliase, including medroxyprogesterone injection, within 3 months prior to screening and during study participation.
- Current use of levodopa.
- Alanine aminotransferase (ALT) concentration ≥ 2 times ULN.
- Creatinine >1.5 times ULN.

Dosing Regimen and Formulation

Pegvaliase was provided either in vial and syringe (VS) or prefilled syringes (PFS). Of the 247 subjects who exclusively used VS or PFS, 134 subjects used VS only and 113 subjects used PFS only.

The pegvaliase dosing regimen for induction, titration, and maintenance periods with the PFS presentation is as follows:

Table 10: Pegvaliase Dosing Schema

Pegvaliase Dosing (Induction, Titration, and Maintenance), Prefilled Syringe						
Study Period	Duration	Total Weekly Fixed Dose (mg)	Total Weekly Volume (mL) [a]	Mg per Dose	Volume (mL) per Dose [a]	Frequency of Administration per Week
Induction	4 weeks	2.5 [b]	0.5	2.5	0.5	1
		2.5 [b]	0.5	2.5	0.5	1
		2.5	0.5	2.5	0.5	1
		2.5	0.5	2.5	0.5	1
Titration	Up to 30 weeks	5	1.0	2.5	0.5	2 [c]
		10	0.5	10	0.5	1
		20	1.0	10	0.5	2 [c]
		40	2.0	10	0.5	4
		70	3.5	10	0.5	7
		140 [d] (20 mg/day)	7.0	20	1.0	7
		280 [d] (40 mg/day)	14.0	40	2.0	7
Maintenance	At least 2 weeks	20 mg/day or 40 mg/day				

[a] Pegvaliase was provided in prefilled syringes in 3 sizes: 2.5 mg (0.5 mL of 5 mg/mL protein concentration), 10 mg (0.5 mL of 20 mg/mL protein concentration), and 20 mg (1.0 mL of 20 mg/mL protein concentration).

[b] Self-administration of at least the first 2 doses of study drug was performed in the clinic.

[c] It was recommended that dosing not be performed on consecutive days (ie, at least 1 day was to occur between the 2 doses when possible).

[d] Subjects randomized to the 20 mg/day or 40 mg/day dose regimen continued with this dose regimen until at least approximately Week 26 and up to Week 36.

Source: Applicant's submission, dated 6/30/2017, Module 5.3.5.2 Synopsis, page 5.

Dose titration was individualized based on tolerability, allowing for dose reductions or interruptions due to AEs. The duration of titration could range from 6 weeks, the minimum amount of time to reach 20 mg/day with no dose interruptions, to 30 weeks, the maximum amount of time to reach 40 mg/day allowing for dose reductions or interruptions as needed. If subjects did not reach the target dose of 20 mg/day or 40 mg/day by Week 34, they maintained the tolerated dose for the last 2 weeks of the trial.

Administration

As stated in the protocol (Applicant's submission, dated 6/30/2017, Module 5.3.5.2 Protocol and Protocol Amendments, pages 77 - 78), "Subjects (or a subject-designated caregiver) must meet the following criteria to be eligible to self-administer the drug:

- Has no known cognitive impairment that may increase the safety risk per investigator assessment
- Has no medical history or is not taking any medications that may compromise self-administration of study drug per investigator assessment
- Has completed all required self-administration training and has demonstrated self-administration competency per investigator assessment, including how to prepare the study drug for administration and safely perform the injection."

As discussed below with the Amendment 2, "A competent adult who is capable of observing and assisting the subject will be present during study drug administration and for a minimum of 1 hour following administration for the first 16 weeks of the study; administration of study drug may only be performed if this person is present."

Diet

Patients were required to maintain a relatively constant protein intake throughout the trial, which was managed by a dietitian, so that changes in Phe can be realiably interpreted. As stated in the protocol (Applicant's submission, dated 6/30/2017, Module 5.3.5.2 Protocol and Protocol Amendments, page 81), "A 3-day diet diary and a nutrient analysis software program (Metabolic Pro®) will be used to establish baseline Phe and protein intake levels prior to Day 1. Protein from medical foods (Phe-free amino acid fortified food sources) and protein from intact foods (any other food sources containing Phe) are collectively referred to as dietary protein." "Subjects will be required to maintain dietary protein intake levels that are consistent with their baseline levels for the entire duration of the study, with a consistent diet defined as one in which the intact protein changes are <10 % from baseline and the medical food protein changes <10% from baseline." If subjects were not able to maintain dietary protein within 10% from baseline, the dietitian counseled and eventually discussed non-adherence with the medical

monitor.

Patients were required to take a tyrosine supplement (tyrosine 500 mg 3 times per day with meals).

Trial Endpoints

Primary Endpoints - Safety:

- AEs, including SAEs
- Clinical laboratory tests (chemistry, hematology, and urinalysis) results
- Vital signs
- Physical examination
- ECG test results
- Immunogenicity test results (total anti-rAvPAL-PEG, anti-rAvPAL IgG, anti-rAvPAL IgM, anti-PEG IgM, anti-PEG IgG, anti-rAvPAL IgE, anti-rAvPAL-PEG IgE, and neutralizing antibodies)

Secondary Endpoint - Efficacy:

- Change from baseline to end of study in blood Phe concentration.

Statistical Analysis Plan

The measurement of efficacy was blood Phe concentration, which was assessed at screening, Day 1, Week 3, Week 4 and then every 4 weeks afterwards till up to Week 36 or end of study. Baseline blood Phe concentration was defined as the average of all blood Phe concentrations collected between the Screening visit and Day 1 before initial dosing. Efficacy analyses were based on the Intent-to-treat (ITT) population which consisted of all subjects who were randomized to study treatment. The blood Phe concentrations were summarized descriptively by visit.

Protocol Amendments

Key changes with Amendment 2, August 18, 2014 (per Applicant's submission, dated 6/30/2017, Module 5.3.5.2 Protocol and Protocol Amendments, pages 2 – 4).

- Revised individual and study stopping criteria
  - "If a severe or life-threatening National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) grade ≥ 3 hypersensitivity event occurs that is treatment-related and meets clinical criteria (Brown, S. G., 2004, J.Allergy Clin.Immunol.) for severe, an ad hoc independent DMC will convene within 7 days of the event to review and advise the sponsor on potential changes to the

study conduct."
    - "Individual subjects who have an NCI-CTCAE grade ≥ 3 hypersensitivity event that is treatment-related and is suspected to meet the clinical criteria (Brown, S. G., 2004, J.Allergy Clin.Immunol.) for severe in the judgment of the investigator and the sponsor's medical monitor may be permanently discontinued from study drug."
    - "Anaphylaxis (per National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network [NIAID/FAAN] criteria) is an AE of special interest that should be reported to the sponsor within 24 hours to facilitate rapid reporting for sponsor review."

- "The study eligibility criteria have been modified to include only subjects who are ≥18 years old."
- Safety precautions for anaphylaxis
    - "Subjects must have a competent adult present during study drug administration and for a minimum of 1 hour following administration for the first 16 weeks of the study."
    - "Premedication with H1 antagonist, H2 antagonist, and non-steroidal anti-inflammatory medication (NSAIDs) is administered approximately 2-3 hours prior to each dose of study drug until completion of dose titration."
    - "At least two epinephrine injectors will be issued to subjects for use in case of anaphylaxis. Subjects will receive training on self-administration and will be instructed to carry one epinephrine injector with them at all times."
- "Anti-rAvPAL immunoglobulin E (IgE) antibody assessment has been added to the Hypersensitivity Reaction Visit to better assess IgE response."

8.1.2 165-301: Trial Results

Compliance with Good Clinical Practices (GCP)

As stated on page 47 of the CSR, "This study was conducted in accordance with the following:

- United States (US) Code of Federal Regulations (CFR) sections that addressed clinical research studies, and/or other national and local regulations, as applicable
- E6 ICH Guideline for GCP
- The ethical principles established by the Declaration of Helsinki

An informed consent form was completed for every subject prior to the start of the study.

Financial Disclosure

The Applicant has adequately disclosed financial interests with clinical investigators. See financial disclosure in the Appendix 16.2 for further information.

Patient Disposition

261 subjects were randomized in study 301. 131 subjects were randomized to the 20 mg/day group and 130 subjects were randomized to the 40 mg/day group. 61.1% (80) subjects in the 20 mg/day group and 55.4% (72) subjects in the 40 mg/day group reached the maintenance period and screened for study 302 Part 1. 19.8% (26) subjects from the 20 mg/day group and 19.2% (25) from the 40 mg/day group did not reach the maintenance period and enrolled in study 302 Part 4 directly. The major reason or study discontinuation and study drug discontinuation were due to adverse event. Details are shown in **Table 11**.

Table 11: Trial 165-301 - Patient Disposition by Randomized Dose Group

End of Study Status	20 mg (N=131)	40 mg (N=130)
Completed Study 301	**111 (84.7%)**	**102 (78.5%)**
reached the maintenance period and screened for Study 302	**80 (61.1%)**	**72 (55.4%)**
screened for Study 302 and enrolled in 302 Part 4 directly	**26 (19.8%)**	**25 (19.2%)**
Maintenance dose not reached	6 (4.6%)	10 (7.7%)
Early closure of Study 302 Part 2	20 (15.3%)	15 (11.5%)
not screened in Study 302	**5 (3.8%)**	**5 (3.8%)**
Completed treatment	3 (2.3%)	1 (0.8%)
Early study drug discontinuation	2 (1.5%)	4 (3.1%)
Early Study Discontinuation	**20 (15.3%)**	**28 (21.5%)**
Adverse event	8 (6.1%)	9 (6.9%)
Death	1 (0.8%)	0
Pregnancy	1 (0.8%)	0
Lost to follow-up	1 (0.8%)	1 (0.8%)
Other	0	2 (1.5%)
Protocol Deviation	0	2 (1.5%)
Study terminated by sponsor	0	1 (0.8%)
Physician decision	3 (2.3%)	2 (1.5%)
Withdrawal by subject	6 (4.6%)	11 (8.5%)

Source: Tables 9.1.4 and 9.1.5 in Study 301 CSR and Reviewer's analysis.

Table 12 shows treatment duration by study period. The median treatment duration of induction period was 28 days for both dose groups. The median treatment duration of the titration period was 42 days for the 20 mg/day group and 56 days for the 40 mg/day group.

The median duration between the first dose of 20 mg and the first dose of 40 mg was 7 days.

Table 12: Trial 165-301 – Treatment Duration (Days) by Study Period

Study Period	20 mg/day	40 mg/day	Overall
Induction			
N	131	130	261
Mean (STD)	30.4 (11.1)	28.6 (7.0)	29.5(9.3)
Median	28	28	28
Q1, Q3	28, 29	28, 28	28, 29
Min, Max	8, 98	1, 78	1, 98
Titration			
N	123	124	247
Mean (STD)	53.2 (24.3)	71.6 (38.6)	62.4 (33.5)
Median	42	56	49
Q1, Q3	42, 56	49, 84	42, 70
Min, Max	5, 154	8, 217	5, 217
Maintenance			
N	103	92	195
Mean (STD)	120.5 (58.0)	102.0 (53.6)	111.8 (56.6)
Median	147	104	124
Q1, Q3	59, 172	49, 150.5	56, 161
Min, Max	14, 191	14, 176	14, 191
Total Treatment Duration			
N	131	130	261
Mean (STD)	175.2 (73.5)	169.1 (71.2)	172.1 (72.3)
Median	194	170	184
Q1, Q3	112, 251	105, 250	111, 250
Min, Max	8, 258	1, 253	1, 258
Number of patients with ≥ 36 weeks	30 (22.9%)	25 (19.2%)	55 (21.1%)
Duration between first dose of 20 mg and first dose of 40 mg			
N	17	97	114
Mean (STD)	59.1 (59.6)	10.9 (9.8)	18.1 (29.7)
Median	35	7	7
Min, Max	3, 185	-3, 58	-3, 185

Source: Reviewer's analysis.

Protocol Violations/Deviations

A major protocol deviation was defined by the Applicant as a departure from the approved study protocol that could have affected the rights, safety or welfare of subjects or the integrity of the data. A minor protocol deviation was defined by the Applicant as a departure from the approved study protocol that had minimum or no impact on the rights, safety or welfare of subjects or the integrity of the data. 59% of subjects had at least one major deviation and 99% of subjects had at least one minor deviation. The most common major and minor protocol deviation was "procedure not done." The number of subjects with different types of protocol deviations was similar between the 20 mg/day and 40 mg/day treatment arms.

Table 13: Protocol deviations- Trial 165-301

Table 14.1.4
Protocol Deviations by Randomized Dose Group
Analysis Population: All Enrolled Subjects

Protocol Deviation Category	20 mg/day (N=131)	40 mg/day (N=130)	Total (N=261)
Total Number of Subjects with At Least One Deviation	131 (100.0%)	130 (100.0%)	261 (100.0%)
Number of Subjects with At Least One Major Deviation	77 (58.8%)	78 (60.0%)	155 (59.4%)
Procedure not done	63 (48.1%)	59 (45.4%)	122 (46.7%)
Dosing irregularity	42 (32.1%)	46 (35.4%)	88 (33.7%)
Eligibility criteria	4 (3.1%)	6 (4.6%)	10 (3.8%)
Out of window	0	2 (1.5%)	2 (0.8%)
Number of Subjects with At Least One Minor Deviation	130 (99.2%)	129 (99.2%)	259 (99.2%)
Procedure not done	121 (92.4%)	124 (95.4%)	245 (93.9%)
Out of window	117 (89.3%)	108 (83.1%)	225 (86.2%)
Dosing irregularity	95 (72.5%)	97 (74.6%)	192 (73.6%)
Failure to withdraw	1 (0.8%)	0	1 (0.4%)
Eligibility criteria	0	2 (1.5%)	2 (0.8%)

Source: Applicant's submission, dated 6/30/2017, Module 5.3.5.2, 301 CSR, page 338.

Per trial protocol, patients should maintain a consistent diet during the study. A consistent diet was defined as one in which changes in protein from intact food were kept within ±10 % of

baseline and protein from medical food changes were within ±10% of baseline. During the trial, 2 patients had a consistent diet. Per the clinical reviewers' suggestion that a Phe-restricted diet be defined as 200 to 1100 mg daily dietary Phe intake (according to ACMG PKU Practice Guidelines), 34 patients (13.0%) maintained a Phe-restricted diet during the trial.

Table 14: Trial 165-301: Proportion of Subjects with Restricted Diet During Study

Dietary change during study 301	Average daily protein intake from medical food (g)	Average daily protein intake from intact food (g)	Consistent Diet	Average daily dietary Phe (mg)
within ±10% change from baseline	25 (9.6%)	8 (3.1%)	2 (0.8%)	n/a
within ±25% change from baseline	37 (14.2%)	35 (61.3%)	11 (4.2%)	n/a
within ±75% change from baseline	86 (33.0%)	160 (61.3%)	55 (21.1%)	n/a
Maintain 200 to 1100 mg Phe daily	n/a	n/a	n/a	34 (13.0%)

Resource: Reviewer's summary. Denominator of the percentage is total number of patients 261.

Table of Demographic Characteristics

Table 15: Demographics by Randomized Dose Group- Trial 165-301

Table 14.1.5
Demographics by Randomized Dose Group
Analysis Population: All Enrolled Subjects

Demographic	20 mg/day (N=131)	40 mg/day (N=130)	Total (N=261)
Age at Enrollment (yrs)			
n	131	130	261
Mean (SD)	30.24 (8.63)	28.05 (8.77)	29.15 (8.75)
Median	29.00	26.00	28.00
Min , Max	16.00 , 52.00	16.00 , 55.00	16.00 , 55.00
16 - < 18	5 (3.8%)	6 (4.6%)	11 (4.2%)
18 - < 66	126 (96.2%)	124 (95.4%)	250 (95.8%)
≥ 66	0	0	0
Sex			
Female	62 (47.3%)	68 (52.3%)	130 (49.8%)
Male	69 (52.7%)	62 (47.7%)	131 (50.2%)
Race			
American Indian or Alaska Native	0	1 (0.8%)	1 (0.4%)
Black or African American	1 (0.8%)	2 (1.5%)	3 (1.1%)
Native Hawaiian or Pacific Islander	0	0	0
White	130 (99.2%)	124 (95.4%)	254 (97.3%)
Other	0	2 (1.5%)	2 (0.8%)
Missing	0	1 (0.8%)	1 (0.4%)
Ethnicity			
Hispanic or Latino	6 (4.6%)	1 (0.8%)	7 (2.7%)
Not Hispanic or Latino	125 (95.4%)	128 (98.5%)	253 (96.9%)
Missing	0	1 (0.8%)	1 (0.4%)

Source: Applicant's submission, dated 6/30/2017, Module 5.3.5.2 CSR for study 301, page 339-340.

The demographics of the Trial 301 population are generally representative of adults with PKU and the population in whom the drug will ultimately be indicated for. For a detailed discussion of demographics and PKU statistics, refer to the section "Adequacy of the safety database" below. The demographics are very similar between the 20 mg and 40 mg treatment groups. Missing data for race and ethnicity is negligible.

Other Baseline Characteristics (e.g., disease characteristics, important concomitant drugs)

Table 16: Baseline Characteristics by Randomized Dose Group

Table 14.1.6
Baseline Characteristics by Randomized Dose Group
Analysis Population: All Enrolled Subjects

Characteristic	20 mg/day (N=131)	40 mg/day (N=130)	Total (N=261)
Weight, kg			
n	131	129	260
Mean (SD)	82.0 (20.49)	78.9 (20.85)	80.5 (20.68)
Median	81.1	74.9	77.2
Min , Max	45.3 , 139.2	41.5 , 135.9	41.5 , 139.2
Height, cm			
n	131	129	260
Mean (SD)	168.1 (9.25)	168.1 (9.68)	168.1 (9.45)
Median	167.6	167.6	167.6
Min , Max	143.5 , 192.0	149.0 , 190.0	143.5 , 192.0
BMI, kg/m2			
n	131	129	260
Mean (SD)	29.0 (6.96)	27.8 (6.48)	28.4 (6.74)
Median	28.5	26.9	27.7
Min , Max	17.1 , 47.3	17.2 , 46.8	17.1 , 47.3
< 25	46 (35.1%)	47 (36.2%)	93 (35.6%)
25-< 30	31 (23.7%)	41 (31.5%)	72 (27.6%)
>= 30	54 (41.2%)	41 (31.5%)	95 (36.4%)
Missing	0	1 (0.8%)	1 (0.4%)

Baseline Blood Phe, umol/L			
n	131	130	261
Mean (SD)	1241.0 (389.70)	1224.4 (384.28)	1232.7 (386.36)
Median	1253.0	1215.5	1221.0
Min , Max	285.0 , 2186.0	483.0 , 2330.0	285.0 , 2330.0
Baseline Protein Intake			
Patients on restricted Diet	22 (16.8%)	19 (14.6%)	41 (15.7%)
Patients taking Protein from Medical Food	75 (57.3%)	74 (56.9%)	149 (57.1%)

Source: Applicant's submission, dated 6/30/2017, Module 5.3.5.2 CSR for study 301, pages 341-345.

The treated patient population is reflective of adults with PKU largely on an unrestricted diet who have poor metabolic control manifesting with a mean baseline blood Phe concentration > 1,200 micromol/L. As such, the trial population represents the patient population that the product is indicated for, specifically, patients with uncontrolled Phe concentrations >600 micromol/L on existing management.

Treatment Compliance, Concomitant Medications, and Rescue Medication Use

As defined on page 100 in the Trial 301 CSR, "The usage rate was derived from the total amount of study drug intake divided by the total dispensed study drug amount over the study period, and multiplied by 100%." As shown in the Applicant's table below, 96 subjects (73.3%) in the 20 mg group and 93 subjects (71.5%) in the 40 mg group administered at least 80% of the dispensed drug. This level of treatment compliance is reasonable.

Table 17: Study Drug Use by Randomized Dose Group

Table 10.3.1: Study Drug Use by Randomized Dose Group (Intent-to-Treat Population)

	20 mg/day (n = 131)	40 mg/day (n = 130)	Total (n = 261)
Total Amount of Study Drug (mg) Received			
n	131	130	261
Mean (SD)	2095.3 (1378.25)	3273.8 (2426.69)	2682.3 (2054.29)
Median	2390.0	3122.5	2615.0
Min, Max	5.0, 3975.0	2.5, 7575.0	2.5, 7575.0
Study Drug Usage			
n	131	130	261
Mean (SD)	86.6% (20.72%)	81.7% (27.40%)	84.2% (24.36%)
Median	94.2%	90.9%	92.0%
Min, Max	10.3%, 170.2%	3.3%, 198.2%	3.3%, 198.2%
≥ 80% Usage	96 (73.3%)	93 (71.5%)	189 (72.4%)

SD, standard deviation.

Study drug usage was calculated as following: 100 x (actual dose)/(total planned dose).

Source: Table 14.3.1.1.1

Source: Applicant's submission, dated 6/30/2017, Module 5.3.5.2 CSR for study 301, page 111.

Efficacy Results – Primary Endpoint

The efficacy evaluation was the secondary objective of the study. See Section for "Efficacy Results – Secondary and other relevant endpoints" for the efficacy results.

Data Quality and Integrity

The submitted datasets and definition files were accessible and the quality of the submitted datasets was acceptable.

Efficacy Results – Secondary and other relevant endpoints

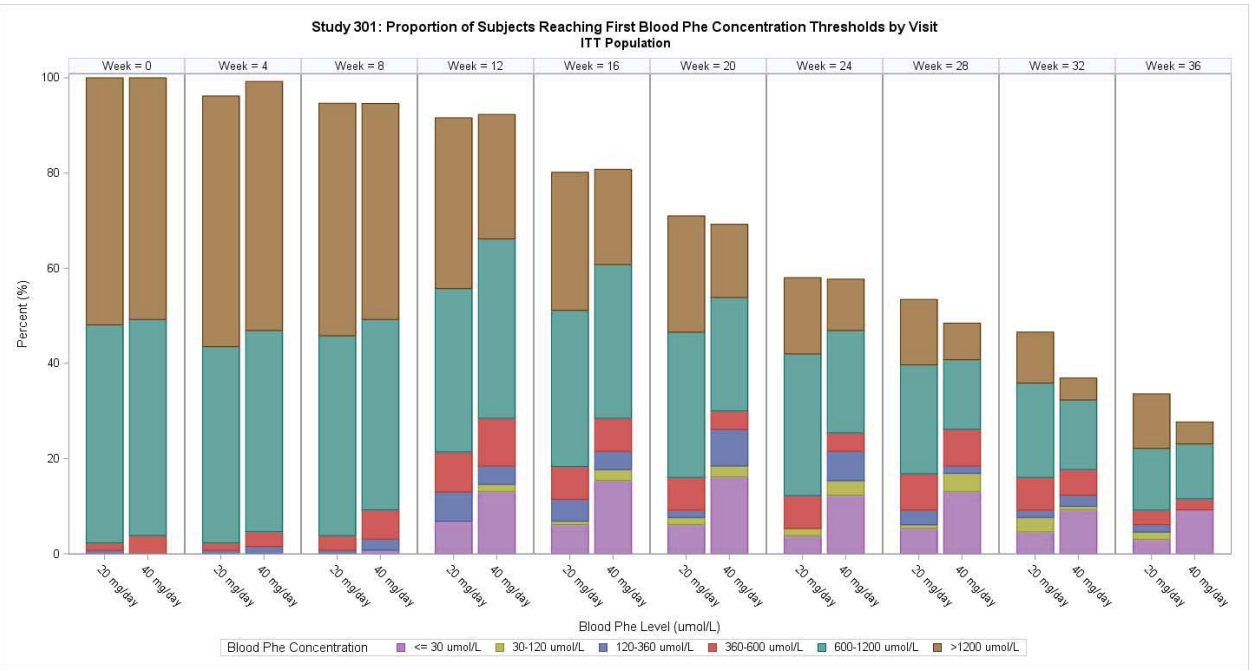
Table 18 presents the blood Phe concentration at each visit in Trial 301 for the ITT population. The subjects' blood Phe concentration was numerically reduced over time for both dose groups. It should be noted that the sample size declined over time as subjects discontinued early or were transitioned to Trial 165-302. The reduction in blood Phe concentration in the 40 mg/day group is numerically greater than that observed in the 20 mg/day group.

Table 18: Trial 165-301: Blood Phe Concentration Over Time - ITT Population

	20 mg/day			40 mg/day		
Visit	N	Mean (SD)	Min, Max	N	Mean (SD)	Min, Max
Baseline	131	1241.0 (389.7)	285, 2186	130	1224.4 (384.3)	483, 2330
Week 8	124	1227.7 (374.6)	242, 2192	123	1168.4 (428.0)	0, 2230
Week 12	120	997.0 (513.84)	0, 1951	120	859.1 (534.1)	0, 2025
Week 16	105	952.7 (493.6)	0, 1997	105	798.7 (563.9)	0, 1948
Week 20	93	933.4 (487.1)	0, 1795	90	677.5 (552.0)	0, 1915
Week 24	76	929.2 (449.0)	0, 1881	75	668.0 (547.9)	0, 1842
Week 28	70	852.9 (500.5)	0, 1818	63	597.8 (535.6)	0, 1649
Week 32	61	800.9 (499.6)	0, 1732	48	585.8 (481.8)	0, 1690
Week 36	44	868.4 (501.8)	0, 1738	36	624.4 (530.6)	0, 1606

Source: Table 10.4.1.1 in 165-301 CSR.

Figure 4: Trial 165-301: Proportion of Subjects Reaching First Blood Phe Concentration Thresholds by Visit - ITT Population



Source: Reviewer's analysis.

Table 19 and **Figure 4** present the proportion of subjects reaching blood Phe reduction thresholds of ≤ 30, 30-120, 120-360, 360-600, 600-1200, > 1200 micromol/L, and having a first 20% reduction from pre-treatment baseline by study visit. Patients began to show blood Phe concentrations in 120-360 micromol/L or 360-600 micromol/L ranges starting at Week 4 of treatment when the dose was 2.5 mg once weekly. From Week 12, more patients showed blood Phe concentration ≤ 30 micromol/L in the 40 mg/day arm compared to the 20 mg/day arm. The incidence of concentrations ≤ 30 micromol/L was 2-fold higher in the 40 mg/day arm compared to the 20 mg/day arm from Week 12 to the end of study.

Table 19: Proportion of Subjects Reaching Pre-Defined First Blood Phe Concentration Thresholds by Study Visit - ITT Population

Randomized Dose Group	Visit	≤30 micromol/L	(30, 120] micromol/L	(120, 360] micromol/L	(360, 600] micromol/L	(600, 1200] micromol/L	>1200 micromol/L	20% reduction from baseline
20 mg (N=131)	Baseline	0	0	1 (0.8%)	2 (1.5%)	60 (45.8%)	68 (51.9%)	0
	Week 3	0	1 (0.8%)	0	3 (2.3%)	60 (45.8%)	65 (49.6%)	17 (13.0%)
	Week 4	0	0	1 (0.8%)	2 (1.5%)	54 (41.2%)	69 (52.7%)	13 (9.9%)
	Week 8	0	0	1 (0.8%)	4 (3.1%)	55 (42.0%)	64 (48.9%)	15 (11.5%)
	Week 12	9 (6.9%)	0	8 (6.1%)	11 (8.4%)	45 (34.4%)	47 (35.9%)	48 (36.6%)

Randomized Dose Group	Visit	≤30 micromol/L	(30, 120] micromol/L	(120, 360] micromol/L	(360, 600] micromol/L	(600, 1200] micromol/L	>1200 micromol/L	20% reduction from baseline
	Week 16	8 (6.1%)	1 (0.8%)	6 (4.6%)	9 (6.9%)	43 (32.8%)	38 (29.0%)	44 (33.6%)
	Week 20	8 (6.1%)	2 (1.5%)	2 (1.5%)	9 (6.9%)	40 (30.5%)	32 (24.4%)	44 (33.6%)
	Week 24	5 (3.8%)	2 (1.5%)	0	9 (6.9%)	39 (29.8%)	21 (16.0%)	41 (31.3%)
	Week 28	7 (5.3%)	1 (0.8%)	4 (3.1%)	10 (7.6%)	30 (22.9%)	18 (13.7%)	40 (30.5%)
	Week 32	6 (4.6%)	4 (3.1%)	2 (1.5%)	9 (6.9%)	26 (19.8%)	14 (10.7%)	35 (26.7%)
	Week 36	4 (3.1%)	2 (1.5%)	2 (1.5%)	4 (3.1%)	17 (13.0%)	15 (11.5%)	20 (15.3%)
40 mg (N=130)	Baseline	0	0	0	5 (3.8%)	59 (45.4%)	66 (50.8%)	0
	Week 3	0	0	2 (1.5%)	9 (6.9%)	53 (40.8%)	62 (47.7%)	23 (17.7%)
	Week 4	0	0	2 (1.5%)	4 (3.1%)	55 (42.3%)	68 (52.3%)	17 (13.1%)
	Week 8	1 (0.8%)	0	3 (2.3%)	8 (6.2%)	52 (40.0%)	59 (45.4%)	20 (15.4%)
	Week 12	17 (13.1%)	2 (1.5%)	5 (3.8%)	13 (10.0%)	49 (37.7%)	34 (26.2%)	61 (46.9%)
	Week 16	20 (15.4%)	3 (2.3%)	5 (3.8%)	9 (6.9%)	42 (32.3%)	26 (20.0%)	64 (49.2%)
	Week 20	21 (16.2%)	3 (2.3%)	10 (7.7%)	5 (3.8%)	31 (23.8%)	20 (15.4%)	57 (43.8%)
	Week 24	16 (12.3%)	4 (3.1%)	8 (6.2%)	5 (3.8%)	28 (21.5%)	14 (10.8%)	46 (35.4%)
	Week 28	17 (13.1%)	5 (3.8%)	2 (1.5%)	10 (7.7%)	19 (14.6%)	10 (7.7%)	42 (32.3%)
	Week 32	12 (9.2%)	1 (0.8%)	3 (2.3%)	7 (5.4%)	19 (14.6%)	6 (4.6%)	30 (23.1%)
	Week 36	12 (9.2%)	0	0	3 (2.3%)	15 (11.5%)	6 (4.6%)	19 (14.6%)

Source: Reviewer's analysis.

In Trial 165-301, 42 (32.1%) subjects in the 20 mg/day arm and 57 (32.8%) subjects in the 40 mg/day arm showed a blood Phe level ≤ 600 micromol/L during the study (**Table 20**). When looking at the actual dose at which subjects had the first occurrence of blood Phe ≤ 600 micromol/L, 32 (24.4%) subjects were on 20 mg/day (2 subjects during the titration period and 30 subjects during the maintenance period) and 33 (25.4%) subjects were on 40 mg/day (7 subjects during the titration period and 26 subjects during the maintenance period).

Table 20: Trial 301: Incidence of First Blood Phe ≤ 600 micromol/L by Actual Dose at Measurement

Study Period	Actual Dose at First Blood Phe ≤ 600 micromol/L	20 mg/day (N=131)	40 mg/day (N=130)
Induction	2.5 mg/day	4 (3.1%)	11 (8.5%)
Titration	10 mg/day	6 (4.6%)	10 (7.7%)
	20 mg/day	2 (1.5%)	2 (1.5%)
	40 mg/day	0	7 (5.4%)
Maintenance	20 mg/day	30 (22.9%)	1 (0.8%)
	40 mg/day	0	26 (20.0%)
Total		42 (32.1%)	57 (32.8%)

Source: Reviewer's analysis.

Table 21: Trial 165-301: Incidence of First 20% Reduction in Blood Phe Concentration by Actual Dose at Measurement

Study Period	Actual Dose at First 20% Reduction in Blood Phe Concentration	20 mg/day (N=131)	40 mg/day (N=130)
Induction	2.5 mg/day	21 (16%)	28 (21%)
Titration	2.5 mg/day	2 (2%)	0
	10 mg/day	12 (9%)	12 (9%)
	20 mg/day	5 (4%)	4 (3%)
	40 mg/day	0	11 (8%)
Maintenance	20 mg/day	**48 (37%)**	0
	40 mg/day	1 (1%)	**36 (27%)**
	40.2 mg/dy	0	2 (2%)
Total		89 (68%)	93 (72%)

Source: Reviewer's analysis.

Dose/Dose Response

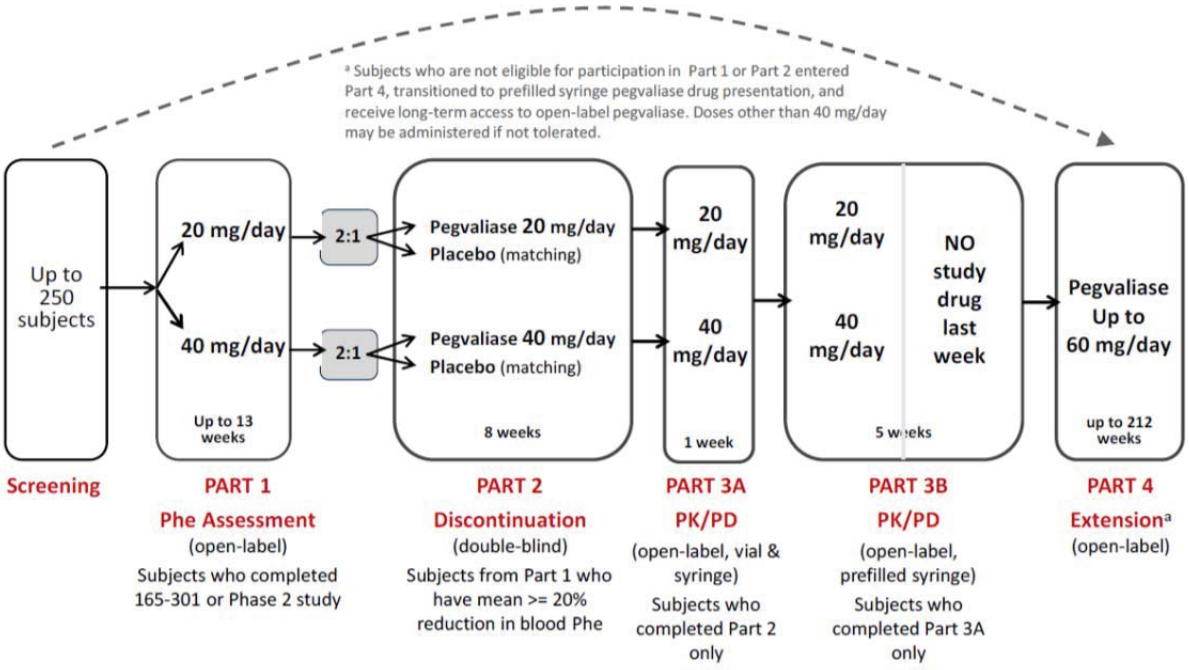
Dose-response was not formally assessed in trial 301. However, in patients who reached their respective target maintenance dose of either 20mg/day or 40mg/day during Trial 301, the median and first quartile percent reduction in blood Phe concentration appeared to be numerically higher in the 40 mg/day group compared to the 20 mg/day group. Refer to section 6 above for more information.

Durability of Response

The durability of response and persistence of effect were not formally assessed in Trial 301.

8.1.3 Trial 165-302: "A Four-Part, Phase 3, Randomized, Double-Blind, Placebo Controlled, Four-Arm, Discontinuation Study to Evaluate the Efficacy and Safety of Subcutaneous Injections of BMN 165 Self-Administered by Adults with Phenylketonuria"

Figure 5: Overview of 165-302 Trial Design



Source: Applicant's submission, dated 6/30/2017, Module 5.3.5.1 302 CSR, page 6.

Objectives

- **Part 1 Blood Phe assessment:** To screen subjects for eligibility for entry into Part 2 of the study and to characterize the safety of pegvaliase in subjects previously exposed to pegvaliase.

- **Part 2 Randomized withdrawal, double-blind, placebo-controlled:**

    - Primary efficacy: To evaluate blood Phe concentration in subjects previously exposed to pegvaliase who were administered pegvaliase (20 or 40 mg/day) compared with those who were administered a matching placebo.

    - Secondary efficacy: To evaluate inattention and mood symptoms in subjects previously exposed to pegvaliase who were administered pegvaliase (20 or 40

mg/day) compared with those who were administered a matching placebo.

- Safety: To evaluate the safety of pegvaliase in subjects previously exposed to pegvaliase who were administered pegvaliase (20 or 40 mg/day) compared with those who were administered a matching placebo.

- **Part 3 Pharmacokinetic (PK)/Pharmacodynamic (PD) Comparability Assessment**
  - To evaluate multiple-dose PK/PD assessments in subjects who were administered pegvaliase.
  - To evaluate PK comparability between pegvaliase vial and syringe (VS) drug presentation and prefilled syringe (PFS) drug presentation.

- **Part 4 Long-term, open-label extension:**
  - To evaluate the long-term effect of multiple dose levels of pegvaliase on blood Phe concentration in subjects who are administered pegvaliase using PFS drug presentation.
  - To characterize long-term inattention, hyperactivity, and mood symptoms in subjects who are administered pegvaliase.
  - To evaluate long-term safety of multiple dose levels of pegvaliase in subjects who are administered pegvaliase using PFS drug presentation.
  - To characterize protein intake from medical food and intact food in subjects who are administered pegvaliase.
  - To characterize the long-term immunogenicity profile of pegvaliase in subjects who are administered pegvaliase using PFS drug presentation.

Trial Centers and Number of Subjects

29 study centers in the United States. 215 subjects enrolled in this study.

Trial Population

Key Inclusion Criteria Have completed a prior BMN 165 study (PAL-003 or 165-301) prior to screening

- Have had a stable BMN 165 dose regimen for at least 14 days prior to screening
- Are at least 18 years of age and no older than 70 years of age at screening (subjects who were <18 years old and were already enrolled into Study 165-301 under Amendment #1 (10JAN2014) could enroll into this study)
- Has identified a competent person(s) who is >18 years of age and who can observe the subject during study drug administration and for a minimum of 1 hour following administration during Part 3, Week 1; Part 4, Week1; if needed upon return to dosing after an AE; if dosing is increased during Part 4; and per investigator determination.
- Have received documented approval from a study dietitian confirming that the subject can maintain their diet in accordance with Section 9.6 (see diet section below).
- If applicable, maintained stable dose of medication for ADHD, depression, anxiety, or other psychiatric disorder for ≥8 weeks prior to enrollment and willing to maintain stable dose throughout study unless a change is medically indicated.

Key Exclusion Criteria

- Use of any medication (except BMN 165) intended to treat PKU, including the use of large neutral amino acids, within 2 days prior to the administration of study drug (Day 1, first dose of BMN 165).
- Have known hypersensitivity to Dextran® or components of Dextran.
- Use or planned use of any injectable drugs containing PEG (except for BMN 165), including medroxyprogesterone injection, within 3 months prior to screening and during study participation
- Current use of levodopa
- Current participation in the Kuvan registry study (PKU Demographics, Outcomes and Safety [PKUDOS]). Patients may discontinue the PKUDOS registry trial to allow enrollment in this study
- Alanine aminotransferase (ALT) concentration at least 2 times the upper limit of Normal
- Creatinine at least 1.5 times the upper limit of normal

Trial Endpoints

The primary efficacy endpoint was the change in blood Phe concentration from Part 2 baseline to Week 8 of Part 2. The primary efficacy analysis was performed after all subjects completed Part 2.

The secondary efficacy endpoints were changes from the Part 2 baseline to Week 8 of Part 2 for the following neurocognitive and neuropsychiatric symptom scores: ADHD RS-IV Inattention Subscale score (Investigator-Rated) for subjects with a baseline (165-301) score >9, ADHD RS-IV Inattention Subscale score (Investigator-Rated) for all subjects, PKU Profile of Mood States (POMS; Self-Rated) Confusion Subscale score, PKU POMS (Self-Rated) Total Mood Disturbance (TMD) score, and POMS TMD (Self-Rated) score.

Statistical Analysis Plan

The safety population for Part 2 consisted of all subjects who were randomized and received any study drug in Part 2. The ITT population consisted of all subjects who were randomized to Part 2 of the study. The modified ITT (mITT) population consisted of all subjects who reached the target dose and were randomized into Part 2 with a mean blood Phe reduction of at least 20% from baseline levels of Trial 165-301 or the phase 2 study in which they initiated pegvaliase. The primary analysis of the primary and the secondary efficacy endpoints were based on the mITT population and by the randomized treatment assignment.

The primary efficacy endpoint was the change from Part 2 Baseline in blood Phe concentration to Part 2 Week 8. Baseline blood Phe concentration is defined as the last available blood Phe collected prior to Part 2 Day 1 dosing. The change from baseline in blood Phe concentration at Part 2 Week 8 were to be compared between 40 mg/day and 40 mg/day Placebo, and between the 20 mg/day and 20 mg/day placebo group, separately, using repeated measures model with treatment group, visit and treatment by visit interaction as factors adjusting for baseline blood Phe.

Data of blood Phe concentration were summarized using descriptive statistics for the mITT population.

Categories for blood Phe concentration of ≤ 30, 30-120, 120-360, 360-600, 600-1200, > 1200 micromol/L by visit in Part 2 were provided.

Protocol Amendments

Key changes in Amendment 3 dated December 15, 2015 (per Applicant's submission, dated 6/30/2017, Module 5.3.5.1 Protocol and Protocol Amendments, pages 2 – 3) are the following:

- "Additional information about subjects who are trying to conceive, are pregnant, and/or breastfeed during the study has been added. Subjects must stop study drug administration if trying to conceive, are pregnant, and/or are breastfeeding."
- **"**The sample size for Part 2 (Randomized Discontinuation [RDT]) of the study has

been adjusted due to limited enrollment opportunity of subjects from the adult PKU patient population in the United States. Approximately 85 subjects will be randomized into Part 2 to ensure a minimum of approximately 72 subjects for inclusion in the primary efficacy population."

Key changes in Amendment 2 dated August 8, 2014 (per Applicant's submission, dated 6/30/2017, Module 5.3.5.1 Protocol and Protocol Amendments, pages 146 – 150) are the following:

- "Part 1 will now be focused on determining eligibility for participation in Part 2, the Randomized Discontinuation (RDT) Period. Experience from the ongoing, long-term, Phase 2 study, PAL-003, has shown that reductions in blood Phe concentration are more substantial as subjects gain more experience with BMN 165."
- "The pharmacokinetic (PK) and pharmacodynamic (PD) assessments to be performed after Part 2 and prior to the long-term, open-label extension (Part 4) are now referred to as Part 3."
- "The individual and study stopping criteria have been revised and reflect a better understanding of the risk/benefit with BMN 165 administration."
    - If a severe or life-threatening National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) grade ≥ 3 hypersensitivity event occurs that is treatment-related and meets clinical criteria (Brown, 2004, J. Allergy Clin. Immunol.) for severe, an ad hoc independent DMC will convene within 7 days of the event to review and advise the sponsor on potential changes to the study conduct."
    - "Individual subjects who have an NCI-CTCAE grade ≥ 3 hypersensitivity event that is treatment-related and is suspected to meet the clinical criteria (Brown, 2004, J. Allergy Clin. Immunol.) for severe in the judgment of the investigator and the sponsor's medical monitor may be permanently discontinued from study drug."
    - "Anaphylaxis (per National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network [NIAID/FAAN] criteria) is an AE of special interest that should be reported to the sponsor within 24 hours to facilitate rapid reporting for sponsor review."
- "Dosing instructions in response to AEs have been modified to allow more flexibility based on additional clinical experience from the Phase 2 studies regarding the BMN 165 safety profile."
- "The study eligibility criteria have been modified to include only subjects who are ≥ 18 years old."
- "Information regarding diet has been modified and information has been added to enable more robust dietary monitoring during BMN 165 administration. Dietitians at the sites will now be responsible for reviewing and documenting approval of a

subject's dietary suitability for inclusion in the study and their ability to maintain diet throughout participation in the study."

- "Safety precautions have been added to the self-administration information. These include a competent adult present during and for a minimum of 1 hour following study drug administration, premedications, and epinephrine injectors issued to subjects."
- "Anti-rAvPAL immunoglobulin E (IgE) antibody assessment has been added to the Hypersensitivity Reaction Visit to better assess IgE response. The anti-protein IgE test serves as a control to determine whether anti-rAvPAL IgE antibody positivity may be missed in anti-rAvPAL-PEG IgE assessment due to potential protein epitope masking by the extensive PEGylation of the BMN 165."

8.1.4 165-302: Trial Results

Compliance with Good Clinical Practices

As stated on page 91 of the study 302 CSR, "This study was conducted in accordance with the following:

- United States (US) Code of Federal Regulations (CFR) that addressed clinical research studies, and/or other national and local regulations, as applicable
- E6 ICH Guideline for GCP
- The ethical principles established by the Declaration of Helsinki"

Each subject provided written, informed consent before any study-related tests or evaluations were performed.

Financial Disclosure

Refer to above financial disclosure section in Trial 301.

Patient Disposition

162 subjects from study 301 and 12 subjects from Phase 2 studies were enrolled in study 302 Part 1. 95 patients were enrolled in Trial 302 Part 2. 86 of them reached 20% reduction in blood Phe reduction from naïve baseline and included in mITT population. Among the 86 subjects, 5 subjects discontinued early due to AEs and one subject withdrew from the study. Details are shown in **Table 22**.

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Table 22: Trial 165-302: Patient Disposition

Study 301 Status			Study 302 Screening			Study 302 Part 1			Study 302 Part 2					Study 302 part 4
Status	**20 mg (N=131)**	**40 mg (N=130)**	**Status**	**20 mg (N=112)**	**40 mg (N=103)**	**Status**	**20 mg (N=86)**	**40 mg (N=78)**	**Status**	**20 mg (N=34)**	**20 mg P (N=15)**	**40 mg (N=32)**	**40 mg P (N=14)**	**N=202**
Completed Study 301, reached the maintenance dose, and screened for Study 302	80 (61.1%)	72 (55.4%)	Screened and Entered Study 302 Part 1	80+6	72+6	**Entered Part 2 reaching 20% reduction**	39+4 (50.0%)	38+5 (55.1%)	**Entered Part 3**	25+3 (82.4%)	13+1 (93.3%)	22+3 (78.1%)	13+0 (92.9%)	80 (39.6%)
									Entered Part 4 due to AE	1+0 (2.9%)	0	1+2 (9.4%)	1+0 (7.1%)	5 (2.5%)
									Early study discontinuation - withdrawal	0	0	1+0 (3.1%)	0	
						Entered Part 2 without reaching ≥20% reduction	6+0 (7%)	3+0 (3.8%)	**Entered Part 3**	5+0 (14.7%)	1+0 (6.7%)	3+0 (9.7%)	0	9 (4.5%)
						Entered Part 4 without reaching ≥ 20% reduction	26+0 (30.2%)	12+0 (15.4%)						37 (18.3%)
						Entered Part 4 due to early closure of Study 302 Part 2	4 (4.7%)	6 (7.7%)						10 (5.0%)
						Entered Part 4 for other reasons	0+2 (2.3%)	6+1 (9.0%)						10 (5.0%)
						Early Study discontinuation in 165-302 Part 1	5+0 (5.8%)	7+0 (9.0%)						
Completed Study 301, screened for Study 302, and enrolled in 302 Part 4 directly	**26 (19.8%)**	**25 (19.2%)**	Screened and Entered Part 4 directly from Study 301	26	25									51 (25.2%)
Completed Study 301, but not screen in Study 302	**5 (3.8%)**	**5 (3.8%)**												
Early Study Discontinuation	**20 (15.3%)**	**28 (21.5%)**												

Notes: (1) The percentage is out of the "N" in the column header; (2) N=131, 130 are number of subjects enrolled in study 301; N=112, 103 are number of subjects screened for study 302; N=86, 78 are number of subjects enrolled in study 302 Part 1; N=34, 15, 32, 14 are number of subjects enrolled in study 302 Part 2; N=202 is the number of subjects enrolled in study 302 Part 4. (3) Numbers after "+" are the number of patients who came from Phase 2 studies. More details are in Table 47 in the Appendix.

Protocol Violations/Deviations

All subjects had at least one protocol deviation and 76% of subjects had at least one major deviation. The most common protocol deviations were "procedure not done" and "dosing irregularity."

Table 23: Protocol deviations-Trial 165-302

Table 14.1.2.1
Summary of Protocol Deviation
Analysis Population: All Subjects Entered 165-302

Protocol Deviation Category	Total (N=215)
Total Number of Subjects with At Least One Deviation	215 (100.0%)
Number of Subjects with At Least One Major Deviation	164 (76.3%)
Procedure not done	137 (63.7%)
Dosing irregularity	111 (51.6%)
Out of window	31 (14.4%)
Eligibility criteria	13 (6.0%)
Excluded con med	4 (1.9%)
Number of Subjects with At Least One Minor Deviation	212 (98.6%)
Procedure not done	209 (97.2%)
Dosing irregularity	198 (92.1%)
Out of window	191 (88.8%)
Eligibility criteria	1 (0.5%)

Source: Applicant's submission, dated 6/30/2017, Module 5.3.5.1, 302 CSR, page 580.

A consistent diet, defined as daily protein intake within ±10% change from baseline, was required per the study protocol. One patient maintained a consistent diet as defined by the protocol during Trial 302. Phe-restricted diet (PKU diet) was not defined in the study protocol.

Phe restriction was defined by the review team as one consisting of 200- 1100 mg Phe daily, as per the updated nutrition management guidelines for PKU.[32] Using this definition, 14 patients (5.1%) maintained a Phe-restricted diet during study 302.

Table 24: Trial 165-302: Proportion of Subjects with Restricted Diet

Dietary change during trial 302	Average daily protein intake from medical food (g)	Average daily protein intake from intact food (g)	Consistent Diet	Average daily dietary Phe (mg)
within ±10% change from baseline	19 (7.0%)	3 (1.1%)	1 (0.4%)	n/a
within ±25% change from baseline	25 (9.2%)	13 (4.8%)	6 (2.2%)	n/a
within ±75% change from baseline	52 (19.0%)	100 (36.0%)	30 (11.0%)	n/a
Maintain 200 to 1100 mg	n/a	n/a	n/a	14 (5.1%)

Resource: Reviewer's summary. Denominator of the percentage is total number of patients 273.

Table of Demographic and Baseline Characteristics

Table 25 below shows the demographic and baseline characteristics for all 215 subjects who enrolled in Trial 302 Part 1 and the 86 subjects included in Part 2. The demographics and patients' baseline characteristics in both parts are generally similar with the following notable differences. The weight and BMI are higher in the 20mg/day placebo group compared to 40 mg/day placebo group, 20mg/day active group, 40 mg/day active group, and all enrolled subjects. This should not influence the efficacy and safety analyses. In addition, the blood Phe concentration at naïve baseline was higher in the 20 mg/day placebo group and 20 mg/day active group with a mean of 1459 and 1450 micromol/L respectively. The blood Phe concentration at pre-treatment baseline in the 40 mg/day placebo and 40 mg/day active group was 1108 and 1185 micromol/L respectively. The 20 mg/day placebo group has 0 patients on restricted protein intake and the 20 mg/day active group has 1 patient on restricted protein intake, which is likely why the blood Phe concentration at naïve baseline is so high. The other groups have 14-17%of subjects on restricted protein intake. The demographics below are generally representative of PKU and the population in whom the drug will ultimately be indicated for. For a detailed discussion of demographics and PKU statistics, refer to the section "Adequacy of the safety database" below.

[32] Singh RH, Cunningham AC, Mofidi S, et al. Updated, web-based nutrition management guideline for PKU: An evidence and consensus based approach. Mol Genet Metab. 2016 Jun;118(2):72-83.

Table 25: Demographic Characteristics

Naive Demographic or Baseline Characteristic	All Enrolled Subjects (N = 215)	mITT Population			
		20 mg/day Active (N=29)	40 mg/day Active (N=29)	20 mg/day Placebo (n = 14)	40 mg/day Placebo (n = 14)
Age at naive baseline, years					
Mean (SD)	29.22 (8.74)	30.72 (9.09)	28.59 (7.61)	30.50 (10.96)	30.00 (10.22)
Median	28.00	29.00	29.00	27.50	25.50
Min, Max	16.00, 55.00	19.00 , 50.00	16.00 , 40.00	19.00, 51.00	18.00, 50.00
16 to < 18 years old [a]	11 (5.1%)	0	3 (10.3%)	0	0
18 to < 66 years old	204 (94.9%)	29 (100.0%)	26 (89.7%)	14 (100.0%)	14 (100.0%)
Sex at naive baseline					
Female	105 (48.8%)	14 (48.3%)	17 (58.6%)	6 (42.9%)	6 (42.9%)
Male	110 (51.2%)	15 (51.7%)	12 (41.4%)	8 (57.1%)	8 (57.1%)
Race at naive baseline					
White	211 (98.1%)	29 (100.0%)	29 (100.0%)	13 (92.9%)	14 (100.0%)
American Indian or Alaska Native	1 (0.5%)	0	0	0	0
Black/African American	2 (0.9%)	0	0	1 (7.1%)	0
Weight at naive baseline, kg [b]					
n	214	29	29	14	13
Mean (SD)	79.3 (21.15)	81.0 (19.90)	76.2 (23.19)	94.0 (27.17)	73.1 (16.49)
Median	75.1	80.2	71.1	87.9	67.7
Min, Max	41.5, 143.0	47.8 , 125.2	42.0 , 135.9	47.4, 143.0	51.8, 108.4
BMI at naive baseline, kg/m^2 [b]	214			14	13
Mean (SD)	27.9 (6.66)	28.4 (6.71)	27.1 (7.05)	32.6 (7.75)	25.6 (4.37)
Median	26.9	27.9	25.1	33.6	24.2
Min, Max	17.1, 46.7	18.8 , 46.3	17.2 , 42.4	19.2, 45.4	18.3, 33.0
< 25	85 (39.5%)	12 (41.4%)	13 (44.8%)	3 (21.4%)	7 (50.0%)
25 to < 30	58 (27.0%)	6 (20.7%)	6 (20.7%)	1 (7.1%)	3 (21.4%)
≥ 30	71 (33.0%)	11 (37.9%)	10 (34.5%)	10 (71.4%)	3 (21.4%)
Missing	1 (0.5%)	0	0		
Blood Phe concentration at naive baseline, micromol/L					
Mean (SD)	1225.6 (378.98)	1450.2 (310.47)	1185.8 (344.00)	1459.1 (354.71)	1108.9 (266.84)
Median	1196.0	1429.0	1098.0	1504.5	1064.5
Min, Max	285.0 [c], 2229.0	982.0 , 2143.0	713.0 , 1898.0	761.0, 2116.0	695.0, 1549.0
Daily protein from intact food at naive baseline, g e					
n	196	26	22	12	14
Mean (SD)	38.4 (27.96)	48.1 (23.36)	36.2 (26.70)	46.5 (40.48)	35.2 (21.02)
Median	29.1	46.1	29.9	28.6	31.0
Min, Max	3.6, 155.3	8.8 , 93.2	4.2 , 121.5	16.9, 155.3	9.6, 64.9
Daily protein from medical food at					

naive baseline, g					
n	196	26	22	12	14
Mean (SD)	27.9 (29.08)	14.3 (24.96)	28.8 (26.30)	21.2 (25.40)	29.8 (25.79)
Median	20.0	0.0	25.0	10.0	27.5
Min, Max	0.0, 120.0	0.0 , 91.2	0.0 , 60.0	0.0, 66.7	0.0, 73.2
Protein intake at naive baseline					
Restricted protein intake, n (%) f	36 (16.7%)	1 (3.4%)	5 (17.2%)	0	2 (14.3%)

[a] Under Amendment #1 (10JAN2014; 7.8.1) of the study protocol, subjects 2: 16 years old were eligible for study participation.

[b] One subject (b) (6)) in the 40 mg/day placebo group did not have weight and height measured at baseline in 165-301.

[c] One subject (b) (6)) had a naive baseline blood Phe level < 600 micromol/L, which was different from the blood Phe level assessed at the time of screening for eligibility into this study.

[e] RDA for total protein for adults in the general population is 0.75 g/kg. For an 80-kg individual, approximately 60 g of daily protein is recommended.

[f] Subjects were considered to be on restricted protein intake if > 75% of total daily protein intake was from medical food. Total daily protein intake was the sum of daily protein intake from medical food and daily protein intake from intact food.

Source: Applicant's submission, dated 6/30/2017, Module 5.3.5.1 CSR for Trial 302, pages 183-185. Trial 302 CSR Table 14.1.3.1, Table 14.1.4.1, Table 14.1.3.5, Table 14.1.4.5. Key portions of tables included.

Other Baseline Characteristics (e.g., disease characteristics, important concomitant drugs)

See above demographic and baseline characteristics section.

Treatment Compliance, Concomitant Medications, and Rescue Medication Use

As defined in the 302 CSR, "the rate of study drug use was calculated as the total amount of study drug used (as reported by subjects in workbooks) divided by the total amount of study drug dispensed" (page 191). Greater than 96% of subjects in the mITT population administered pegvaliase or placebo, so treatment compliance was very good. The rate of study drug use was similar across treatment arms. This reviewer feels that this rate of study drug use is reasonable given the known risk for immunogenicity and tolerability difficulty.

Efficacy Results – Primary Endpoint

The primary efficacy endpoint of Trial 165-302, part 2, was the change in blood Phe concentration from Part 2 Baseline to Part 2 Week 8. A mixed-model with repeated measures was used to compare the mean change in blood Phe concentration from Part 2 baseline to Part 2 Week 8 between the 20 mg/day and 20 mg/day placebo arms, and between the 40 mg/day and 40 mg/day placebo arms. Both active arms maintained blood Phe significantly better than their corresponding placebo with p-values less than 0.0001. The difference in LS means in the 20 mg/day arm versus placebo comparison was numerically higher than for the 40 mg/day arm versus placebo comparison. Details are shown in **Table 26** and

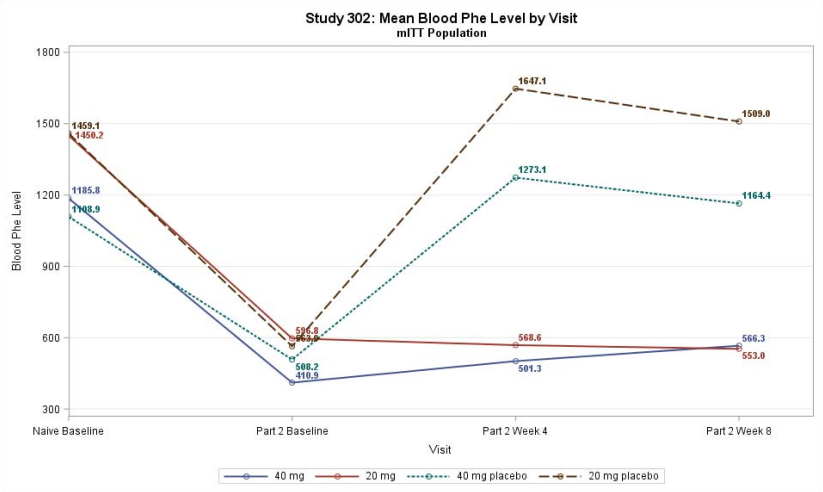
Figure 6.

Table 26: Trial 165-302: Mixed-Model Repeated Measures of Change in Blood Phe Concentration (micromol/L) from Part 2 Baseline to Part 2 Week 8 (mITT Population)

	20 mg/day	20 mg/day Placebo	40 mg/day	40 mg/day Placebo
N	29	14	29	14
Part 2 Baseline Mean (SD)	596.8 (582.7)	563.9 (504.6)	410.9 (439.9)	508.2 (363.7)
Part 2 Week 8 Mean (SD)	553.0 (582.4)	1509.0 (372.6)	566.3 (567.5)	1164.4 (343.3)
Mean (SD) Change from Part 2 Baseline	-65.9 (192.0)	996.4 (555.0)	114.1 (332.4)	599.0 (507.4)
LS Mean Change from Part 2 Baseline (95% CI)	-23.3 (-156.2, 109.7)	949.8 (760.4, 1139.1)	76.3 (-60.2, 212.8)	664.8 (465.5, 864.1)
Difference in LS Means (95% CI)	-973.0 (-1204.2, -741.9)		-588.5 (-830.1, -346.9)	
p-value*	<0.0001		<0.0001	

Source: adapted from Table 14.2.2.1.2 in 165-302 CSR.
* P-value was compared between the active dose group and the according placebo group respectively.

Figure 6: Trial 165-301 and 165-302: Blood Phe Concentration (micromol/L) changes from pre-treatment baseline (165-301) to Part 2 week 8 (165-302)- mITT Population



Source: adapted from Table 9.4.1.3.1 in 165-302 CSR.

The Applicant specified sensitivity analyses using multiple imputations and last observation carried forward. The efficacy results from these sensitivity analyses were consistent with the primary analysis and not included in this review.

Data Quality and Integrity

The submitted datasets and definition files were accessible and the quality of the datasets were acceptable.

Efficacy Results – Secondary and other relevant endpoints

The Agency had previously expressed concerns regarding the use of the selected clinical outcome assessment (COA) scales (ADHD-RS, POMS) to capture relevant changes in attention and mood in adults with PKU owing to psychometric limitations of the instruments as proposed (see Type C meetings on September 30, 2014 and August 18, 2015). In addition, the reported score changes (from Part 2 baseline to Part 2 week 8 in Trial 165-302) in the scales of inattention and mood between patients on pegvaliase and those on placebo were not statistically significant. This may be due to the small sample size and the short duration of treatment in Part 2 (8 weeks). Therefore, no additional analyses were conducted due to the aforementioned limitations.

Dose/Dose Response

There appeared to be a dose-response for blood Phe concentration in those subjects who had dose escalation in Part 4 of the study. In subjects who dose titrated from 20 mg/day to 40 mg/day, the mean (SD) blood Phe concentration decreased from 1,042 (454) micromol/L prior to dose titration (i.e., Week 0) to 552 (516) micromol/L at Week 4 after dose titration in Part 4 of 165-302. Refer to Section 6 for more information.

Durability of Response (Continuous Treatment in Trials 165-301 and 165-302)

Out of 285 patients enrolled in Trial 165-301, there were 118 with baseline blood Phe > 600 micromol/L who were randomized to and received at least one pegvaliase maintenance dose of 20 mg once daily. In this cohort, 52% achieved a single blood Phe measurement of ≤ 600 micromol/L and 30% achieved blood Phe ≤ 600 micromol/L for 16 consecutive weeks (while in the open-label extension of Trial 165-302).

Of 118 patients from Trial 165-301 with a pre-treatment baseline blood Phe concentration > 600 micromol/L who were randomized to and received at least one dose of pegvaliase 20 mg once daily, 53 patients reached their first response (at least a 20% reduction in blood Phe concentration from pre-treatment baseline or a blood Phe concentration ≤ 600 micromol/L) by 4 weeks of treatment with 20 mg once daily and 28 patients reached their first response between Weeks 4 and 24 with 20 mg once daily. Of the 118 patients, 25 patients escalated their dosage from 20 mg once daily to 40 mg once daily before reaching a first response; of those 25 patients, 8 patients reached their first response by 4 weeks of treatment with 40 mg once daily and 6 patients reached their first response between Weeks 4 and 16 with 40 mg once daily.

Table 27: Summary of Subjects Reaching First Response Over Time for Subjects Who Randomized to and Reached 20 mg/day in Study 301 with Baseline Phe > 600 micromol/L

	Number of subjects on study treatment	With ≥ 20% blood phe reduction from naïve baseline or blood phe concentration ≤ 600 micromol/L
Subjects who remained on Treatment		
At 24 weeks	118	
At 48 weeks	81	
At 96 weeks	51	
Subjects who reached first response while on 20 mg/day		
By 4 weeks		53
Between Week 4 and Week 24		28
Between Week 24 and Week 32		0

	Number of subjects on study treatment	With ≥ 20% blood phe reduction from naïve baseline or blood phe concentration ≤ 600 micromol/L
Number of subjects whose dose escalated to 40 mg/day from 20 mg/day		
Reached first response while on 20 mg/day		65
Not reach first response while on 20 mg/day		25
Subjects who did not reach first response on 20 mg/day and reached it once titrated to 40 mg/day		
By 4 weeks		8
Between Week 4 and Week 16		6

Source: Table 15.25 in the applicant response label-updates.pdf submitted on 5/15/2018.

Additional Analyses Conducted on the Individual Trial

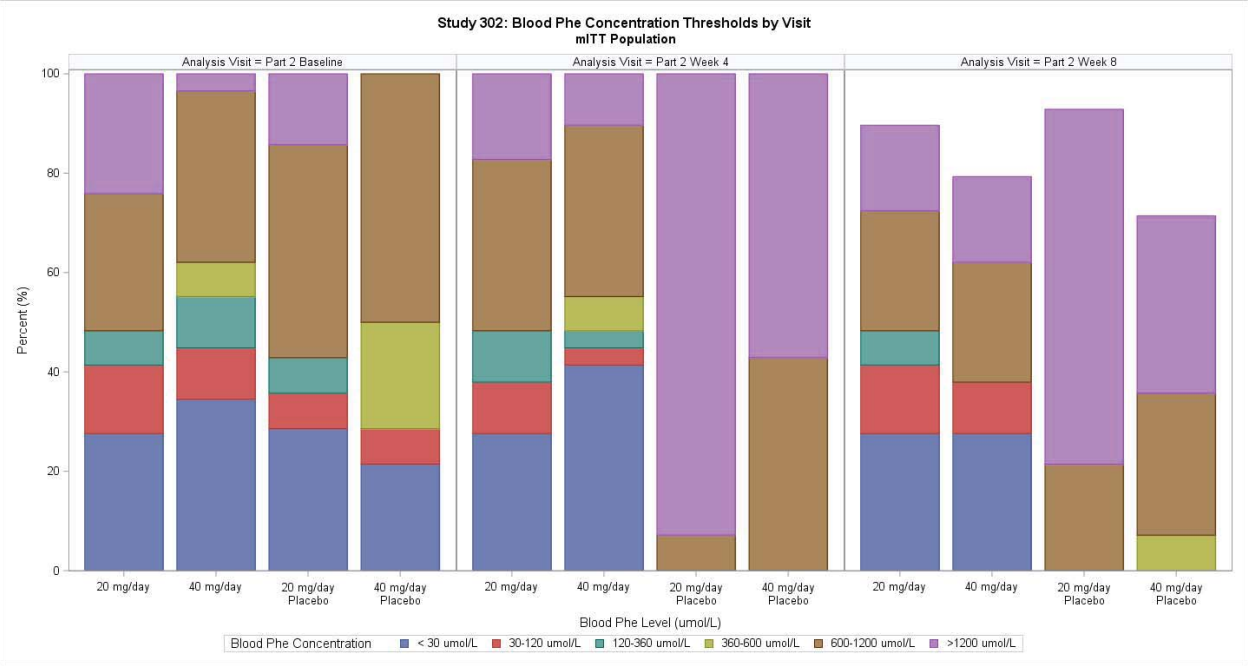
A supportive analysis of number (%) of subjects with blood Phe concentration of ≤ 30, 30-120, 120-360, 360-600, 600-1200, > 1200 micromol/L, and 20% reduction from naïve baseline is presented in Table 28 and Figure 7. As shown, at Part 2 Baseline, the highest responses were in ≤ 30 micromol/L and 600-1200 micromol/L categories, and the response rates were similar among the 4 dose groups. After switching to the placebo groups at Part 2 Week 4 and Week 8, the response rates were greater in the 600-1200 and >1200 micromol/L categories. Response rates in the 20 mg/day and 40 mg/day groups remained consistent during Part 2.

Table 28: Trial 165-302: Blood Phe Concentration Ranges by Visit – mITT Population

Randomized Dose Group	Visit	≤30 micromol/L	30-120 micromol/L	120-360 micromol/L	360-600 micromol/L	600-1200 micromol/L	>1200 micromol/L	20% reduction from naïve baseline
20 mg/day (N=29)	Part 2 Baseline	8 (27.6%)	4 (13.8%)	2 (6.9%)	0	8 (27.6%)	7 (24.1%)	27 (93.1%)
	Part 2 Week 4	8 (27.6%)	3 (10.3%)	3 (10.3%)	0	10 (34.5%)	5 (17.2%)	26 (89.7%)
	Part 2 Week 8	8 (27.6%)	4 (13.8%)	2 (6.9%)	0	7 (24.1%)	5 (17.2%)	23 (79.3%)
40 mg/day (N=29)	Part 2 Baseline	10 (34.5%)	3 (10.3%)	3 (10.3%)	2 (6.9%)	10 (34.5%)	1 (3.4%)	27 (93.1%)
	Part 2 Week 4	12 (41.4%)	1 (3.4%)	1 (3.4%)	2 (6.9%)	10 (34.5%)	3 (10.3%)	23 (79.3%)
	Part 2 Week 8	8 (27.6%)	3 (10.3%)	0	0	7 (24.1%)	5 (17.2%)	17 (58.6%)
20 mg/day Placebo (N=14)	Part 2 Baseline	4 (28.6%)	1 (7.1%)	1 (7.1%)	0	6 (42.9%)	2 (14.3%)	11 (78.6%)
	Part 2 Week 4	0	0	0	0	1 (7.1%)	13 (92.9%)	1 (7.1 %)
	Part 2 Week 8	0	0	0	0	3 (21.4%)	10 (71.4%)	1 (7.1%)
40 mg/day Placebo (N=14)	Part 2 Baseline	3 (21.4%)	1 (7.1%)	0	3 (21.4%)	7 (50.0%)	0	12 (85.7%)
	Part 2 Week 4	0	0	0	0	6 (42.9%)	8 (57.1%)	0
	Part 2 Week 8	0	0	0	1 (7.1%)	4 (28.6%)	5 (35.7%)	2 (14.3%)

Source: CSR Table 14.2.8.1.230, and reviewer's analysis.

Figure 7: Trial 302: Blood Phe Concentration Ranges by Visit



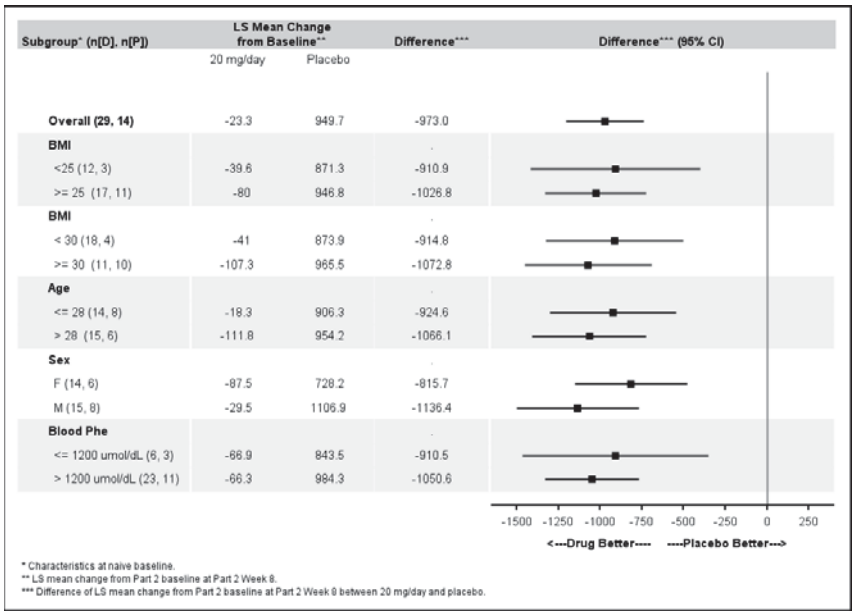
Source: Reviewer's analysis.

Subgroup analysis:

Figure 8 and
Figure 9 present the results for the primary efficacy endpoints (mean change from Part 2 Baseline in blood Phe concentration at Week 8) by BMI, age, sex, and pre-treatment baseline blood Phe concentration for the 20 mg/day and 40 mg/day arms, respectively. Male patients responded numerically better than female patients in both the 20 mg/day and 40 mg/day arms while the two confidence intervals for the treatment effect overlap. The treatment effect was

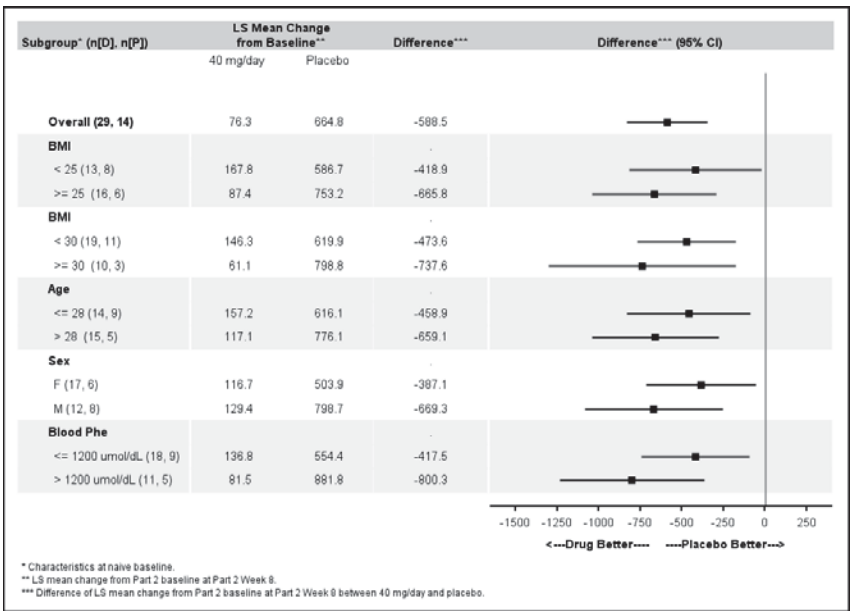
generally consistent within subgroups.

Figure 8: Trial 165-302: Mean Blood Phe Change from Part 2 Baseline to Week 8 by Demographic Subgroups– mITT Population (20 mg once daily vs Placebo)



Source: Reviewer's analysis.

Figure 9: Trial 165-302: Mean Blood Phe Change from Part 2 Baseline to Week 8 by Demographic Subgroups– mITT Population (40 mg once daily vs Placebo)



Source: Reviewer's analysis.

8.1.5 Assessment of Efficacy Across Trials

Efficacy was assessed across Trials 165-301 and 165-302 which represented a continuum of treatment for many patients. Patients treated in Trial 165-301 enrolled and received further treatment in Trial 165-302. The evidence for primary efficacy was provided during treatment in Trial 302 part 2 (randomized withdrawal period). Of 118 patients from Trial 165-301 with a pre-treatment blood Phe concentration > 600 micromol/L who received at least one dose of pegvaliase 20 mg once daily, 81 patients (70%) reached their first response (at least a 20% reduction in blood Phe concentration from pre-treatment baseline or a blood Phe concentration ≤ 600 micromol/L) between 4 weeks and 24 weeks of treatment with 20 mg once daily (which occurred during either Trial 165-301 or Trial 165-302). Of the 118 patients, 25 patients (22%) increased their dose to 40 mg once daily while in Trial 165-302 part 4, and, of those, 14 patients (56%) achieved a therapeutic response after 4-16 weeks of additional treatment with 40 mg once daily.

8.1.6 Integrated Assessment of Efficacy

The Applicant has demonstrated that pegvaliase reduces blood Phe concentrations through an induction, titration, and maintenance regimen in Trial 165-301. In Part 2 of Trial 165-302, an 8-week, placebo-controlled, randomized withdrawal trial, both dosages of pegvaliase (20 mg once daily and 40 mg once daily) maintained the patients' blood Phe concentrations as compared to placebo patients whose blood Phe concentrations returned to pre-treatment levels after 8

weeks of treatment withdrawal. This difference was statistically significant. The magnitude of blood Phe reduction in the pegvaliase trials was variable among patients owing to the immune responses to the product which affected the therapeutic response. The efficacy results as presented by the Applicant and confirmed by the review team provide substantial evidence that pegvaliase at a maintenance dose of 20 mg once daily and up to a maximum of 40 mg once daily reduces blood Phe concentrations in adults with PKU on an unrestricted diet to levels that are at the generally recommended therapeutic goal of ≤ 600 micromol/L.

8.2 Review of Safety

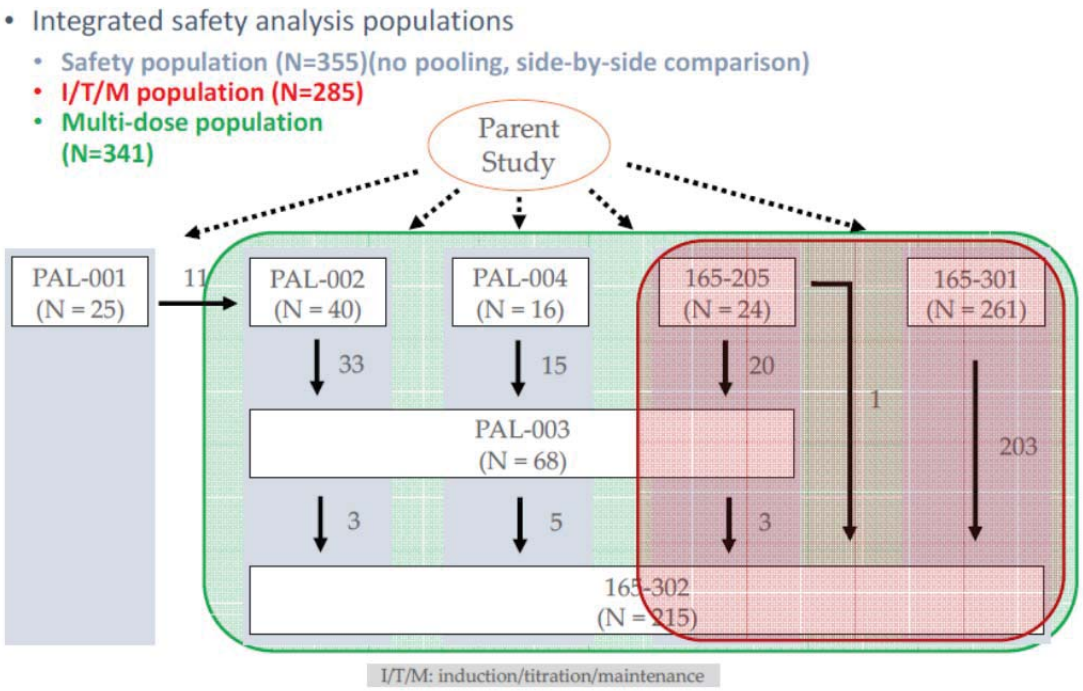
8.2.1 Safety Review Approach

The focus of the safety review is the Induction/Titration/Maintenance (I/T/M) population (n=285), which includes all subjects treated with pegvaliase in an I/T/M dosing regimen as proposed in the product's label. This I/T/M population is the most clinically relevant to inform prescribers and patients on the product's safety. The I/T/M population includes subjects from the parent Trials 165-205 and 165-301 and their feeder Trials PAL-003 and 165-302.

In addition to the I/T/M population, the Applicant submitted similar safety analyses for the Multiple Dose (MD) population (n=341), which includes all subjects who received more than one dose of pegvaliase, regardless of the dosing regimen, and excludes the 14 subjects in the Phase 1 Trial PAL-001. However, the trial designs and dosing regimens were different among clinical trials in the program and some Phase 2 trials were not reflective of how the drug is intended to be administered (I/T/M dosing). Across different trials, the periods of fixed vs. adjustable dosing varied, as did the dosed used and frequency of dosing. The MD population could potentially capture rare AEs in studies with relatively small numbers of subjects by including more subjects than the I/T/M population. However, the incidence and event rates for the AEs of special interest/significance, as defined by the Applicant, were generally very similar between the I/T/M and MD populations. As such, the safety review focuses on the I/T/M population (highlighted in yellow in the below tables). Safety results were similar in the MD population.

The Applicant's pooling of the subjects for safety analyses are shown below with the I/T/M population highlighted in red.

Figure 10: Pooling of Subjects for Safety Analyses



Source: Applicant's Clinical Response to Information Request, Sequence 26, dated 11/9/2017, Module 1.11.3, question 13 (A), page 32.

In the I/T/M dosing regimen there were two distinct periods, the induction/titration period when the dose was changing and the maintenance period when the dose was stable. The rates of AEs and laboratory abnormalities varied significantly between the periods and because patients were in these periods for different lengths of time based on tolerability, the team felt it was important to separate the data by period. The subjects in the induction/titration period were treatment naïve while those subjects in the maintenance period were non-treatment naïve, and the safety profiles varied based on the high immunogenicity of the drug. Because of these differences, the AE and laboratory data are presented by treatment period in this review and product labeling. Presenting safety manifestations by period is most clinically meaningful, so prescribers and patients can better anticipate potential complications depending on which treatment period the patient is in at a particular time.

The induction/titration period was defined as the time prior to reaching a stable dose. A stable dose was defined as completing an 8-week duration of treatment with at least an 80% compliance rate at the same dose level. Once the subjects completed 8 weeks on a stable dose, they were considered to have reached the maintenance period. As agreed upon with the Applicant, for subjects who reached the maintenance period, safety data were included in the induction/titration or maintenance periods depending on the AE onset date. Subjects who did not reach the maintenance period were included in the induction/titration period.

Dose adjustments were allowed in the maintenance period. These adjustments could occur due to an AE, hypophenylalanemia, or during Part 4 of Trial 165-302. In Part 4 subjects who were on 20 mg/day were to increase to 40 mg/day at the start and could further increase from 40 mg/day to 60 mg/day. Thus, there was a range of doses in the maintenance period and, to determine the dose response, the team felt it was necessary to analyze the AE and laboratory data by dose on or prior to AE onset within the maintenance period.

For the AE and laboratory data tables, the team included the incidence (number of subjects with percentage) to give a general idea of how many subjects experienced at least one AE in a given category. The tables also include the exposure-adjusted event rate to allow for comparison between the periods given that subjects were on different doses for different lengths of time.

A major safety signal identified with pegvaliase is the high hypersensitivity rate, including anaphylaxis. This was a major focus of the safety review, including coding and characterizing all anaphylactic events, and critically analyzing all elements of the proposed Risk Evaluation and Mitigation Strategy (REMS) for anaphylaxis risk.

8.2.2 Review of the Safety Database

Overall Exposure

The Applicant states that, "In the I/T/M Population, a total of 285 subjects have had an overall exposure of 579.6 patient-years as of the data cutoff" per the May 6, 2017, safety update. "The mean (SD) duration of exposure for the I/T/M Population is 24.40 (15.46) months." The breakdown of the number of subjects by duration of treatment is shown in the table below. Of note, in the I/T/M population, the number of patients treated significantly decreases to n=137 (48.1%) and n= 85 (29.8%) at ≥ 2 years and ≥3 years, respectively. Long-term safety data should be interpreted with this limitation, since only approximately half of the subjects had exposure to treatment ≥ 2 years and even fewer subjects had exposure of ≥ 3 years and ≥ 4 years. Also shown in the table below is the contribution from the two individual trials (165-205 and 165-301) which make up the I/T/M population, with the majority of subjects from Trial 165-301.

Table 29: Cumulative Extent of Exposure by Parent Study

Table 5.1.1.2: Cumulative Extent of Exposure by Parent Study (Safety Population)

	Parent Study (Includes Data from All Subsequent Studies)						
	PAL-001 (n=25)	PAL-002 (n=40)	PAL-004 (n=16)	165-205 (n=24)	165-301 (n=261)	I/T/M[a] (n=285)	MD[b] (n=341)
Duration of treatment with study drug (months)[c]							
n	25	40	16	24	261	285	341
Mean (SD)	0.03 (0.00)	47.12 (33.58)	54.81 (22.62)	39.76 (19.56)	22.99 (14.27)	24.40 (15.46)	28.49 (20.95)
Median	0.03	50.15	66.25	50.22	22.74	23.36	24.90
Min, Max	0.0, 0.0	1.6, 91.4	0.2, 70.6	1.7, 59.3	0.0, 47.2	0.0, 59.3	0.0, 91.4
Subjects by duration of treatment, n (%)	25 (100.0%)	40 (100.0%)	16 (100.0%)	24 (100.0%)	261 (100.0%)	285 (100.0%)	341 (100.0%)
≥ 6 months	0	31 (77.5%)	15 (93.8%)	21 (87.5%)	208 (79.7%)	229 (80.4%)	275 (80.6%)
≥ 1 year	0	30 (75.0%)	15 (93.8%)	21 (87.5%)	188 (72.0%)	209 (73.3%)	254 (74.5%)
≥ 2 years	0	26 (65.0%)	14 (87.5%)	18 (75.0%)	119 (45.6%)	137 (48.1%)	177 (51.9%)
≥ 3 years	0	24 (60.0%)	12 (75.0%)	16 (66.7%)	69 (26.4%)	85 (29.8%)	121 (35.5%)
≥ 4 years	0	21 (52.5%)	12 (75.0%)	13 (54.2%)	0	13 (4.6%)	46 (13.5%)
Total number of days dosed (days)							
n	25	40	16	24	261	285	341
Mean (SD)	1.0 (0.0)	789.7 (726.3)	1,367.4 (621.5)	903.8 (516.2)	599.5 (408.4)	625.1 (425.9)	679.3 (505.2)
Median	1.0	634.0	1,601.0	971.0	600.0	614.0	633.0
Min, Max	1, 1	8, 2,210	5, 1,974	16, 1,671	1, 1,363	1, 1,671	1, 2,210
Total amount of study drug received (mg)							
n	25	40	16	24	261	285	341
Mean (SD)	2.0 (2.6)	36,099.7 (46,724.6)	39,269.8 (28,399.8)	31,018.2 (25,582.6)	20,306.4 (16,727.1)	21,208.5 (17,833.5)	23,802.7 (24,214.7)
Median	0.7	24,828.2	31,401.2	22,461.8	19,435.0	19,585.0	20,336.4
Min, Max	<1, 8	6, 255,614	20, 100,346	80, 82,072	3, 64,805	3, 82,072	3, 255,614

	Parent Study (Includes Data from All Subsequent Studies)						
	PAL-001 (n=25)	PAL-002 (n=40)	PAL-004 (n=16)	165-205 (n=24)	165-301 (n=261)	I/T/M[a] (n=285)	MD[b] (n=341)
Average daily dose over treatment duration (mg/day)[d]							
n	25	40	16	24	261	285	341
Mean (SD)	2.0 (2.6)	19.2 (19.5)	22.7 (12.8)	23.4 (15.7)	23.6 (13.0)	23.5 (13.3)	23.0 (14.1)
Median	0.7	16.1	20.7	23.4	26.8	26.3	25.6
Min, Max	<1, 8	<1, 103	4, 48	2, 50	<1, 51	<1, 51	<1, 103
Average daily dose received (mg/day)[e]							
n	25	40	16	24	261	285	341
Mean (SD)	2.0 (2.6)	38.5 (32.0)	28.2 (16.3)	32.6 (21.0)	28.6 (13.0)	28.9 (13.9)	30.0 (17.3)
Median	0.7	33.5	27.1	28.7	31.9	31.8	31.7
Min, Max	<1, 8	1, 150	4, 59	5, 67	3, 54	3, 67	1, 150

I/T/M, Induction, Titration, Maintenance (Population); Max, maximum; MD, Multiple Dose (Population); Min, minimum; SD, standard deviation.

The Safety Population included all enrolled subjects who received at least one dose of pegvaliase in any of the pegvaliase studies.

The parent study was the first study in which a subject was enrolled. For subjects who continued pegvaliase in Study PAL-003 and/or Study 165-302, data from all enrolled studies were included.

[a] Subjects were from Study 165-205 or Study 165-301 where pegvaliase administration was initiated as an induction, titration, and maintenance dosing regimen.

[b] Subjects who were only enrolled in the Phase 1, single-dose study, PAL-001, were excluded.

[c] Time was months from the first dose to the last dose administered across all studies in which a subject was enrolled. Intervals of missing doses that were > 28 consecutive days were excluded from the calculation of treatment duration.

[d] Calculated by the total amount of study drug received / the duration of treatment in days.

[e] Calculated by the total amount of study drug received / the total number of days dosed.

Source: Table 2.7.4.2.1

Source: Applicant's submission, dated 10/31/2017, Module 5.3.5.3 120-Day Safety Update, Table 5.1.1.2: Cumulative Extent of Exposure by Parent Study (Safety Population), pages 79-80.

Relevant characteristics of the safety population:

As reported by the Applicant and summarized in the Table below, the subjects were about half males and half females, median age of the subjects at enrollment was 27 years, and the majority were White. The trial population is representative of the indicated population with discussion in the "Adequacy of the safety database" section below.

Table 30: Demographics by Parent Study

Table 1.3.1.1: Demographics by Parent Study (Safety Population)

	Parent Study (Includes Data from All Subsequent Studies)					I/T/M[a] (N=285)	MD[b] (N=341)
	PAL-001 (n=25)	PAL-002 (n=40)	PAL-004 (n=16)	165-205 (n=24)	165-301 (n=261)		
Sex, n (%)							
Male	15 (60.0%)	20 (50.0%)	3 (18.8%)	11 (45.8%)	131 (50.2%)	142 (49.8%)	165 (48.4%)
Female	10 (40.0%)	20 (50.0%)	13 (81.3%)	13 (54.2%)	130 (49.8%)	143 (50.2%)	176 (51.6%)
Age at Baseline (years)[c]							
n	25	40	16	24	261	285	341
Mean (SD)	28.4 (7.4)	26.1 (6.4)	32.2 (8.3)	29.3 (11.4)	29.1 (8.7)	29.2 (9.0)	28.9 (8.7)
Median	30.0	25.0	32.0	24.5	28.0	27.0	27.0
Min, Max	18, 43	16, 43	18, 50	16, 56	16, 55	16, 56	16, 56
Subjects by age group, n (%)							
16 - < 18 years	0	2 (5.0%)	0	1 (4.2%)	11 (4.2%)	12 (4.2%)	14 (4.1%)
18 - < 65 years	25 (100.0%)	38 (95.0%)	16 (100.0%)	23 (95.8%)	250 (95.8%)	273 (95.8%)	327 (95.9%)
Ethnicity, n (%)							
Hispanic or Latino	0	1 (2.5%)	0	0	7 (2.7%)	7 (2.5%)	8 (2.3%)
Not Hispanic or Latino	25 (100.0%)	39 (97.5%)	16 (100.0%)	24 (100.0%)	253 (96.9%)	277 (97.2%)	332 (97.4%)
Missing	0	0	0	0	1 (0.4%)	1 (0.4%)	1 (0.3%)

	Parent Study (Includes Data from All Subsequent Studies)					I/T/M[a] (N=285)	MD[b] (N=341)
	PAL-001 (n=25)	PAL-002 (n=40)	PAL-004 (n=16)	165-205 (n=24)	165-301 (n=261)		
Race, n (%)							
American Indian or Alaska Native	0	1 (2.5%)	0	0	1 (0.4%)	1 (0.4%)	2 (0.6%)
Asian	1 (4.0%)	1 (2.5%)	0	0	0	0	1 (0.3%)
Black or African American	0	0	0	0	3 (1.1%)	3 (1.1%)	3 (0.9%)
White	24 (96.0%)	38 (95.0%)	16 (100.0%)	24 (100.0%)	254 (97.3%)	278 (97.5%)	332 (97.4%)
Other	0	0	0	0	2 (0.8%)	2 (0.7%)	2 (0.6%)
Missing	0	0	0	0	1 (0.4%)	1 (0.4%)	1 (0.3%)

I/T/M, induction, titration, maintenance; Max, maximum; Min, minimum; SD, standard deviation

The Safety Population included all enrolled subjects who received at least one dose of pegvaliase in any of the pegvaliase studies.

The parent study was the first study in which a subject was enrolled. For subjects who continued pegvaliase in PAL-003 and/or 165-302, data from all enrolled studies were included.

[a] Subjects were from 165-205 or 165-301 where pegvaliase administration was initiated as an induction, titration, and maintenance dosing regimen.

[b] Subjects who were only enrolled in the Phase 1, single-dose study, PAL-001, were excluded.

[c] At the time of enrollment to subject's parent study.

Source: ISS Table 2.7.4.1.3

Source: Applicant's submission, dated 6/30/2017, Module 5.3.5.3 Integrated Summary of Safety, Table 1.3.1.1: Demographics by Parent Study (Safety Population), pages 58 and 59.

In addition to demographics, additional important characteristics of the I/T/M population are shown below.

Table 31: Blood Phe Concentration (micromol/L) at Pre-Treatment Baseline

Naive Demographic or Baseline Characteristic	Parent Study				I/T/M Population [a] (n = 285)	Multiple-Dose Population (N = 341)
	PAL-002 (n = 40)	PAL-004 (n = 16)	165-205 (n = 24)	165-301 (n = 261)		
n	40	16	24	261	285	341
Mean (SD)	1310.8 (353.9)	1482.1 (363.5)	1168.8 (291.0)	1232.7 (386.4)	1227.3 (379.3)	1249.1 (379.1)
Median	1364.5	1447.5	1093.0	1221.0	1201.0	1238.0
Min, Max	249, 1878	968, 2214	713, 2021	285, 2330	285, 2330	249, 2330
≤ 600 µmol/L	1 (2.5%)	0	0	8 (3.1%)	8 (2.8%)	9 (2.6%)
> 600 to ≤ 900 µmol/L	5 (12.5%)	0	3 (12.5%)	47 (18.0%)	50 (17.5%)	55 (16.1%)
> 900 µmol/L	34 (85.0%)	16 (100.0%)	21 (87.5%)	206 (78.9%)	227 (79.6%)	277 (81.2%)

Source: Applicant's submission, dated 6/30/2017, Module 2.7.3, Summary of clinical efficacy, Table 2.7.3.3.1.1.1: Demographics and Baseline Characteristics (I/T/M Population and Multiple-Dose Population), pages 56-57.

Adequacy of the safety database:

The size of the I/T/M population of 285 subjects with exposure of 579.6 patient-years appears adequate for the purposes of the BLA review and in the context of a rare disease such as PKU. Overall, this population appears representative of the adult PKU population and the patients who would be treated with pegvaliase in clinical practice. "In the United States, PKU is most

common in people of European or Native American ancestry. It is much less common among people of African, Hispanic, or Asian ancestry."[33] On the basis of 10 years of Maryland newborn screening data, the frequency of PKU in African Americans was one-third that in whites.[34] The I/T/M population was almost exclusively White. There is a slight overrepresentation of the white race and underrepresentation of other races, but not to the extent that it could skew the results. No genotype data were submitted with this application. Due to the autosomal recessive pattern of inheritance, males and females are equally affected, and this is seen in the I/T/M population.

As stated in the ACMG practice guidelines, "While many individuals with PAH deficiency do not receive psychiatric disorder diagnoses, elevated rates of psychiatric symptoms, especially anxiety and depression, are common."[35] The prevalence and severity of psychiatric problems generally correlate with the timing and degree of exposure to elevated blood Phe levels, and so rates vary in the literature.[36] The higher incidence rates of psychiatric disorders was reflected in the I/T/M population, in which 123 subjects (43.2%) reported at least one psychiatric disorder as part of their medical history. These included anxiety (23.5%), depression (18.6%), attention deficit/hyperactivity disorder (8.8%), insomnia (6.3%) and panic attack (3.5%). Psychiatric medical history is adequately represented in the safety database.

The baseline severity of disease of the subjects in the I/T/M population is reflective of uncontrolled PKU patients with mostly classical PKU. The naïve baseline mean (SD) blood Phe concentrations was 1,227.3 µmol/L (379.3). These subjects are uncontrolled on existing management and represent the target population with Phe levels significantly higher than the life-long goal of Phe levels between 120-360 micromol/L per the ACMG guidelines.

[33] https://www.nichd.nih.gov/health/topics/pku/conditioninfo/Pages/risk.aspx
[34] Hofman KJ, Steel G, Kazazian HH, Valle D. Phenylketonuria in U.S. blacks: molecular analysis of the phenylalanine hydroxylase gene. Am J Hum Genet. 1991 Apr;48(4):791-8.
[35] Vockley J, Andersson HC, Antshel KM et al. Phenylalanine hydroxylase deficiency: diagnosis and management guideline. *Genet Med*. 2014 Feb;16(2):188-200.
[36] Brumm VL, Bilder D, Waisbren SE. Psychiatric symptoms and disorders in phenylketonuria. Mol Genet Metab. 2010;99 Suppl 1:S59-63.

8.2.3 Adequacy of Applicant's Clinical Safety Assessments

Issues Regarding Data Integrity and Submission Quality

There were no concerns about data quality and integrity. The datasets were accessible with analytic tools and there was appropriate use of standard terminology. The variables were populated by expected data points.

Categorization of Adverse Events

Treatment-emergent adverse events (TEAEs) were defined as "any event which newly appeared or worsened in severity after initiation of pegvaliase dosing and up to 30 days after the completion of dosing." This reviewer considers this definition appropriate.

AEs were coded with Medical Dictionary for Regulatory Activities (MedDRA) (version 18.0). AE severity was graded using Common Terminology Criteria for Adverse Events (CTCAE), with the version specified within each study protocol. The Applicant used consistent clinical laboratory parameters. As stated in the ISS, "where possible, National Cancer Institute Common Terminology Criteria (NCI-CTC) laboratory grades were applied to laboratory values (some laboratory parameters do not have NCI-CTC laboratory grading scales) and are included in the NCI-CTC shift tables. When laboratory parameters did not have NCI-CTC grading criteria, laboratory values were assessed as abnormal low or abnormal high and are included in abnormality shift tables."

Laboratory values of interest were analyzed, including those reported by the investigators as AEs and changes in laboratory as defined by the laboratory criteria. In the Sponsor's IR

response on 3/19/2018, the following terms in the laboratory abnormalities tables were laboratory abnormalities considered clinically significant by the investigator and reported as AEs: C-reactive protein increased, complement factor C3/C4 decreased, alanine aminotransferase increased, and blood creatine phosphokinase increased. However, the team felt that objective criteria should be used when reporting these laboratory abnormalities, especially since causality can be difficult to determine in the absence of a control arm. During labeling negotiations, the Sponsor was asked to objectively define laboratory abnormalities in the PI, (b) (4)

Routine Clinical Tests

See Appendix under "Other Information" for a schedule of assessments for Trial 301 and Trial 302 Part 2. Clinically relevant tests were collected at appropriate time intervals, including 12 lead ECGs, vital signs, weight and height, laboratory tests (chemistry, hematology, urinalysis, immunogenicity, urine pregnancy, plasma Phe and tyrosine), and neurocognitive assessments. These efficacy and safety assessment methods and time intervals are reasonable for the studied population and indication that was investigated.

8.2.4 Safety Results

The following outlines the review team's grouping of preferred terms (PT) in the safety review which differs from the Applicant's submitted grouping of PTs and their initially submitted safety analyses. The following tables use the team's PT grouping which was discussed and agreed-upon with the Applicant during the review cycle and is also reflected in the prescribing information.

Group	Includes PTs
Injection site reaction (MedDRA high level term)	Injection site reaction, injection site erythema, injection site pruritus, injection site pain, injection site bruising, injection site rash, injection site swelling, injection site urticaria, injection site induration, injection site hemorrhage, injection site edema, injection site mass, injection site inflammation, injection site nodule, injection site discoloration, injection site warmth, injection site hematoma, injection site irritation, injection site vesicles, injection site hypersensitivity, injection site papule, injection site discomfort, injection site scar, injection site paresthesia, injection site hypertrophy, injection site extravasation, injection site dryness
Arthralgia (Applicant defined)	Arthralgia, back pain, musculoskeletal pain, pain in extremity, and neck pain.
Hypersensitivity (modified narrow SMQ with FDA adjudicated anaphylaxis/anaphylactoid reactions)	Rash, urticaria, rash generalized, hypersensitivity, rash erythematous, anaphylactic reaction, rash maculo-papular, rash pruritic, serum sickness, swelling face, dermatitis contact, swollen tongue, lip swelling, anaphylactoid reaction, rash macular, pharyngeal edema, injection site hypersensitivity, eczema, drug eruption, dermatitis allergic, dermatitis,

	tongue edema, palatal edema, edema mouth, multiple allergies, lip edema, eye edema, exfoliative rash, drug hypersensitivity, dermatitis atopic, dermatitis acneiform, pruritus allergic, mouth swelling, implant site rash, gingival swelling, face edema, eyelid edema, eye swelling, dermatitis psoriasiform, dermatitis infected, conjunctivitis allergic, bronchospasm, angioedema, allergic sinusitis, allergic cough
Generalized skin reaction lasting at least 14 days (Applicant defined)	Pruritus, rash, urticaria, dry skin, rash erythematous, erythema, cellulitis, rash macular, petechiae, dermatitis allergic, skin infection, skin induration, rash maculo-papular, rash generalized, pruritus generalized, pharyngeal edema, macule, granulomatous dermatitis, exfoliative rash, drug eruption, dermatitis atopic, dermatitis, xanthogranuloma, skin plaque, skin mass, skin lesion, skin hypopigmentation, skin hypertrophy, skin hyperpigmentation, skin exfoliation, septal panniculitis, scleroderma, scar, rash pruritic, rash papular, psoriatic arthropathy, pruritus allergic, papule, necrobiosis lipoidica diabeticorum, furuncle, eczema, ecchymosis, dermatitis psoriasiform, dermatitis infected, blister,
Abdominal pain (Applicant defined)	Abdominal pain, abdominal pain upper, abdominal discomfort
Headache	Headache, migraine, sinus headache
Sinusitis	Sinusitis, sinus congestion

Deaths

There was one death in the clinical development program, occurring in Trial 165-301. This subject (b) (6) was a firefighter who was fatally electrocuted while on his ladder truck carrying a water hose. The investigator reported this event as not related to pegvaliase treatment and this reviewer agrees with the Applicant's assessment.

Serious Adverse Events

Tables 30 and 31 below show the incidence and exposure-adjusted event rates of serious AEs (SAEs) by treatment period. For the I/T/M subjects who reached the maintenance period, the SAEs are broken down by dose on or prior to AE event onset in the maintenance period. The numbers of SAEs are low (≤ 4 events), except for hypersensitivity, which includes FDA adjudicated anaphylaxis. The hypersensitivity SAEs are probably drug related due to the product's immunogenicity. These are expected SAEs given that pegvaliase is a foreign protein derived from bacteria. Based on details from the narratives, timing, investigator assessment, and the nature of the drug, these SAEs are probably drug related.

For hypersensitivity SAEs, the exposure adjusted event rate was higher in the induction/titration period and significantly declined in the maintenance period. The frequent hypersensitivity SAEs in the induction/titration period correlate with the early immune response when ADA and CIC increased and peaked, and complements C3 and C4 dropped to

the lowest levels. There were no trends observed when comparing the doses in the maintenance period given the small numbers of SAEs as shown in Table 33 below. Of note, the exposure-adjusted event rate was approximately equal between the 20mg/day and 40mg/day arms for hypersensitivity SAEs and was very low.

The team considered all FDA adjudicated anaphylaxis events to be SAEs as they were all life-threatening. It is not clear why the Applicant chose to upgrade some of the anaphylaxis and anaphylactoid AEs to SAEs and not others. Refer to section 8.2.5 for further discussion of anaphylaxis and FDA internal adjudication of all anaphylaxis cases. The FDA-adjudicated anaphylaxis results are listed after the SAE table in Table 34 below.

The 6 SAEs of increased blood CPK in 5 subjects presented a possible safety signal. The narratives were reviewed and pertinent details are included in the Appendix. This reviewer feels that these SAEs of elevated blood CPK are likely not pegvaliase induced. They were all reported by the investigators as unrelated to pegvaliase treatment. They all had an alternative explanation of increased strenuous physical exercise close to the time of the lab abnormalities. The increased CPK levels normalized in all the subjects without treatment, except for subject (b) (6) who had mildly elevated CPK levels with a possible ongoing intense exercise program. At this time based on these five subjects, this reviewer is not concerned about a safety signal although routine pharmacovigilance is recommended in the postmarketing setting.

Of the three anxiety events in subjects (b) (6) and (b) (6) only one event was reported as related to study drug by the investigator. Causality is difficult to definitively determine as all three subjects had a history of anxiety, depression, or post-partum depression. In addition, there were other stressors such as school, work, or family. There was also anxiety centered around the study, such as concern about randomization to the placebo arm and blinding during Part 2 of Trial 165-302 or administering the pegvaliase injections. There were other possible explanations for symptoms, such as hypokalemia in subject (b) (6) or allergic reaction in subject (b) (6). So, given these circumstances it is hard to tease out the impact of pegvaliase on these anxiety events since patients with PKU may have manifestations of anxiety and other psychiatric illnesses.

Similar to anxiety, causality is hard to establish for the single depression SAE because the subject had a medical history of depression and other stressors. This subject ((b) (6)) was having hypophenylalaninemia at the time of the depressive episode and the investigator reported the event as related to treatment with pegvaliase. In subjects with pre-existing psychiatric illness, it is difficult to determine what is related to PKU, drug induced, due to independent stressors, or multi-factorial.

There were 2 SAEs identified as being generalized skin reactions lasting ≥ 14 days in the maintenance period. One subject ((b) (6)) developed skin exfoliation during Part 4 of Trial 165-302. The dermatologist's impression from the narrative was "erythematous eruption with

limited desquamation and bulla in acral/flexural distribution < 10% body surface involvement. No significant mucosal involvement was noted, and the subject was otherwise stable. A skin biopsy on the foot lesion demonstrated a cell-poor subcorneal blister with no inflammatory response and minimal fibrosis in the dermis. The pathologist concluded that the histologic features of the biopsy were non-specific but the most likely diagnosis was post-streptococcal desquamation. The lack of inflammatory cells on light microscopy was inconsistent with Stevens-Johnson syndrome. Results of a PAS stain and Gram stain were negative for microorganisms, and an anti-streptolysin O titre was also negative." Also reported in the narrative was that "because the subject was using tampons for menstruation prior to the onset of symptoms, the investigator stated that the most likely explanation for the presentation was staphylococcal scalded skin syndrome/toxic shock syndrome, although no staphylococcal organisms were identified." Treatment for the event included white soft paraffin, acetaminophen, loratadine, ceftriaxone and sulfamethoxazole (for a urinary tract infection per narrative), hydroxyzine, and topical mupirocin. The dose on day of event onset was 40 mg, which was withheld until her condition improved. She was restarted at 10 mg/day and she remained in the study at this dose. The investigator assessed the event as unrelated to pegvaliase. This reviewer agrees that the subject's symptoms could be due to staphylococcal scalded skin syndrome/toxic shock syndrome secondary to tampon use based on the temporal association.

Subject (b) (6) was identified as being generalized skin reaction lasting ≥ 14 days, but following medical review by the Applicant was identified to be an injection site skin reaction lasting ≥ 14 days. This subject injected in the thighs and developed persistent injection site reactions including edema, pruritus and pain. Per the narrative, the "subject received a dermatology consultation and underwent a punch biopsy at the right thigh lesion site. The results of the biopsy revealed histologic features resembling necrobiosis lipoidica diabeticorum, with an initial diagnosis of granulomatous dermatitis. Staining of the biopsy was negative. On 16 December 2014 (during Trial Part 4), she was diagnosed with serious Grade 2 necrobiotic xanthogranuloma-like reaction." The subject withdrew from study drug as a result of this AE and received treatment with adalimumab. "It was reported that her xanthogranuloma had improved, though not dramatically; it became a little softer and lighter after starting adalimumab treatment." The subject stopped treatment with adalimumab when the event resolved after 638 days. This SAE appears to be related to the study drug.

Table 32: Incidence and Exposure-adjusted Event Rate of SAEs by Treatment Period (> 1 Event in either Treatment Period)

Table 1: Incidence and Exposure-Adjusted Event Rate of Serious Adverse Events (SAEs) by Treatment Phase With > 1 Event (2 or More Events) in Either Treatment Phase Analysis Population: I/T/M (N = 285)

	Induction/Titration Phase N = 285 Treatment Duration Mean = 178 day; Median = 116 days; Range: 1 to 1607 days Exposure duration 135.4 person-years		Maintenance Phase N = 223 Treatment Duration Mean = 739 day; Median = 697 days; Range: 5 to 1561 days Exposure duration 444.1 person-years	
Adverse Events by SMQ/HLT/PT	Incidence [Number of Subjects (%)]	Exposure-Adjusted Event Rate [Number of Events/ Person-Year]	Incidence [Number of Subjects (%)]	Exposure-Adjusted Event Rate [Number of Events/ Person-Year]
Skin				
Generalized skin reaction lasting ≥ 14 Days (Sponsor defined) a	0	0	2 (1%)	2 (<0.01)
Immunologic				
Hypersensitivity [b]	16 (6%)	18 (0.13)	8 (4%)	8 (0.02)
Psychiatric				
Anxiety	1 (<1%)	1 (0.01)	2 (1%)	2 (<0.01)
General				
Chest pain	0 (0%)	0 (0)	2 (1%)	3 (0.01)
Non-cardiac chest pain	0 (0%)	0 (0)	2 (1%)	3 (0.01)
Infections				
Appendicitis	0 (0%)	0 (0)	2 (1%)	2 (<0.01)
Investigations				
Blood creatinine phosphokinase increased	2 (1%)	2 (0.01)	3 (1%)	4 (0.01)
Injury, poisoning and procedural complications				
Road traffic accident	0 (0%)	0 (0)	2 (1%)	2 (<0.01)

AESI, adverse event of special interest; FDA, Food and Drug Administration; HLT, high-level term; I/T/M, Induction/Titration/Maintenance; MedDRA, Medical Dictionary for Regulatory Activites; SMQ, Standardized MedDRA Query; PT, preferred term.

Treatment duration is defined as the duration from the first dose administered to last dose administered within each treatment phase.

Exposure-adjusted event rate used the exposure duration (person-years) from first dose administered to last dose administered within each treatment phase. Intervals of missing doses that were > 28 days were excluded. Maintenance Phase was defined as when subjects reached stable dose for 8 weeks. AEs for subjects receiving placebo during Maintenance Phase included.

[a] Terms reported include: skin exfoliation and xanthogranuloma.

[b] Terms reported include: anaphylaxis (per FDA adjudication), serum sickness, hypersensitivity and angioedema. Derived using hypersensitivity modified narrow SMQ with FDA adjudicated Anaphylaxis; injection site rash, injection site urticaria, anaphylactic reaction, and anaphylactoid reaction were excluded from the hypersensitivity narrow SMQ.

Source: Table 15.1 (M1.11.3 SN0056); Table 16.3.1; Table 16.4.1; Listing 16.4.1.1

Source: Applicant's submission, dated 4/10/2018, Module 1.11.3 Response to Request for Clinical Information Dated 26Mar2018, Table 1: Incidence and Exposure-Adjusted Event Rate of Serious Adverse Events (SAEs) by Treatment Phase With > 1 Event (2 or More Events) in Either Treatment Phase Analysis Population: I/T/M (N = 285), pages 3 – 4.

Analysis of exposure-adjusted event rates of SAEs in the maintenance period by dose was similar across all doses.

Table 33: Incidence and Exposure-Adjusted Event Rate of SAEs with > 1 Event in the Maintenance Period

Table 2: Incidence and Exposure-Adjusted Event Rate of Serious Adverse Events (SAEs) With > 1 Event (2 or More Events) in the Maintenance Phase (N = 223)

Adverse Events by SMQ/HLT/PT	Maintenance Phase (N = 223)					
	Dose on or Prior to Time of Adverse Event Onset					
Number of Subjects with Event (%) Number of Events (event rate/person-year)	Placebo (N = 28)	> 0 and < 20 mg/day (N = 52)	20 mg/day [a] (N = 164)	40 mg/day [b] (N = 197)	60 mg/day (N = 82)	Any Dose Level (N = 223)
Total treatment exposure, person-years	4.2	46.2	104.3	234.7	54.6	444.1
Total exposure, days						
Mean (SD)	55 (10.9)	325 (372.4)	232 (217.3)	435 (303.7)	243 (276.2)	727 (372.5)
Median	56	190	195	408	165	690
Min, Max	11 , 64	1 , 1512	1 , 1128	2 , 1185	1 , 875	5 , 1561
Skin						
Generalized skin reaction lasting ≥ 14 Days (Sponsor defined) [c]	0	0	0	2 (1.0%) 2 (0.01)	0	2 (0.9%) 2 (<0.01)
Immunologic						
Hypersensitivity [d]	0	0	2 (1.2%) 2 (0.02)	6 (3.0%) 6 (0.03)	0	8 (3.6%) 8 (0.02)
Psychiatric						
Anxiety	0	0	0	2 (1.0%) 2 (0.01)	0	2 (0.9%) 2 (<0.01)
General						
Non-cardiac chest pain	0	0	0	2 (1.0%) 3 (0.01)	0	2 (0.9%) 3 (0.01)
Chest pain	0	0	0	2 (1.0%)	0	2 (0.9%)
				3 (0.01)		3 (0.01)
Infections						
Appendicitis	0	1 (1.9%) 1 (0.02)	0	1 (0.5%) 1 (<0.01)	0	2 (0.9%) 2 (<0.01)
Investigations						
Blood creatinine phosphokinase increased	1 (3.6%) 1 (0.24)	1 (1.9%) 1 (0.02)	1 (0.6%) 2 (0.02)	0	0	3 (1.3%) 4 (0.01)
Injury, poisoning and procedural complications						
Road traffic accident	0	1 (1.9%) 1 (0.02)	0	1 (0.5%) 1 (<0.01)	0	2 (0.9%) 2 (<0.01)

AESI, adverse event of special interest; FDA, Food and Drug Administration; HLT, high-level term; I/T/M, Induction/Titration/Maintenance; MedDRA, Medical Dictionary for Regulatory Activites; SMQ, Standardized MedDRA Query; PT, preferred term.

Total exposure (days) and total treatment exposure (person-years) used the aggregated duration (person-year) of treatment across all subjects with each dose level. Intervals of missing doses that were > 28 days were excluded. Maintenance Phase was defined as when subjects reached stable dose for 8 weeks. AEs for subjects receiving placebo during Maintenance Phase included.

[a] Exposure duration on 20 mg/day dose included ≥ 20 mg/day to < 40 mg/day doses.

[b] Exposure duration on 40 mg/day dose included ≥ 40 mg/day to < 60 mg/day doses.

[c] Terms reported include: skin exfoliation and xanthogranuloma.

[d] Terms reported include: anaphylaxis (per FDA adjudication), hypersensitivity and angioedema. Derived using hypersensitivity modified narrow SMQ with FDA adjudicated Anaphylaxis; injection site rash, injection site urticaria, anaphylactic reaction, and anaphylactoid reaction were excluded from the hypersensitivity narrow SMQ.

Source: Table 15.2 (M1.11.3 SN0056); Table 16.3.2; Table 16.4.2; Listing 16.4.1.1

Source: Applicant's submission, dated 4/10/2018, Module 1.11.3 Response to Request for Clinical Information Dated 26Mar2018, Table 2: Incidence and Exposure-Adjusted Event Rate of Serious Adverse Events (SAEs) With > 1 Event (2 or More Events) in the Maintenance Phase (N = 223), pages 5 – 6.

Table 34: Incidence of Anaphylaxis- I/T/M Population (N=285)

	DPARP adjudication	Sponsor's internal assessment	Sponsor's external adjudication
Number of subjects, n (%)	26 (9.1%)	33 (11.6)	13 (4.6)
Number of events	37	50	21

A comparison of SAEs in treatment arms versus placebo arms in Trial 165-302, Part 2, did not show significant differences in incidence likely due to the short duration of treatment as shown in Table 35.

Table 35: Incidence of SAEs in Trial 302, Part 2 (8 weeks)

SAE	20 mg/day Active (n=34)	40 mg/day Active (n=32)	20 mg/day Placebo (n=15)	40 mg/day Placebo (n=14)
Agitation	0	1 (3.1%)	0	0
Depression	0	1 (3.1%)	0	0
Blood CPK increased	0	0	1 (6.7%)	0

Source: Applicant's submission, dated 6/30/2017, Module 5.3.5.1 165-302 Study Report Body, Table 14.3.1.4.15 Summary of Incidence of Serious Adverse Event by SOC and Preferred Terms Analysis Population: Part 2 Safety Population 165-302 Part 2 Data, page 3880.

Dropouts and/or Discontinuations Due to Adverse Events

Table 36 and **37** below show the incidence and exposure-adjusted event rates of AEs leading to study drug discontinuation by treatment period and by dose administered during the maintenance. The review team agrees with the Applicant's conclusions that the exposure-adjusted event rate of AEs leading to study drug discontinuation was generally higher during the induction/titration period compared to the maintenance period. In addition, the largest difference in the incidence of AEs leading to discontinuation between the induction/titration

and maintenance periods was observed for hypersensitivity events and arthralgias. The incidence of other AEs was similar between periods.

There was only one patient who discontinued treatment due to proteinuria. This patient was reviewed by our nephrology colleagues. This subject ((b) (6)) was a 19-year-old female with no history of kidney disease who had intermittent mildly elevated UACR values to a maximum of 175 mg/g on Day 148. As stated in the nephrology consult, "around that time, she also reported an AE of urticaria. C3 and C4 were below baseline. No other events suggesting immune complex disease were reported." The subject's UACR was normal the following day after discontinuing pegvaliase. Her serum creatinine remained at her baseline. Per our nephrology colleagues, this narrative does not suggest an immune complex-mediated glomerulonephritis as she did not develop progressive or persistent proteinuria or marked change in renal function.

There was one patient who discontinued due to lymphoid tissue hyperplasia. This subject ((b) (6)) had a Grade 3 SAE. She had a history of diffuse large B-cell non-Hodgkin's lymphoma in 2008 and was in remission since. Per the narrative, "the oncologist noted that activation of her disease after 5 years in remission was unusual, and that the lymphadenopathy might have been unrelated to her history of lymphoma." The subject had a left axillary node biopsy which showed "morphologic and histochemical findings consistent with reactive lymph node hyperplasia in a mixed pattern consisting of follicular and paracortical hyperplasia with focal sinus hyperplasia. There was no evidence of involvement by a neoplasm or other lymphoproliferative process." "The report noted that the paracortical hyperplasia seen in this lymph node could be consistent with a hypersensitivity reaction to pegvaliase." Pegvaliase was permanently discontinued in this subject. Based on the pathology report and high risk of hypersensitivity with pegvaliase treatment, this reviewer feels it is likely that lymphoid tissue hyperplasia is drug induced.

Subject (b) (6) discontinued treatment due to feeling "out of it" and being unresponsive to his name. He received 10 mg of pegvaliase on the day of the AE. The subject also had non-serious Grade 1 dizziness which was described as lightheadedness. As reported in the narrative, "both events resolved later that day without treatment, but pegvaliase was permanently discontinued as a result of the events." This patient's baseline Phe level was 1499 µmol/L and his Phe level 3 weeks prior to AE onset was 1143 µmol/L with study drug dose of 2.5 mg/day. Based on the timing of the drug and symptom onset, as well as no other causal factors described in the narrative, it is likely that pegvaliase was related to the AEs that resulted in drug discontinuation.

Table 36: Incidence and Exposure-Adjusted Event Rate of AEs Leading to Study Drug Discontinuation by Treatment Period (I/T/M population)

Table 3: Incidence and Exposure-Adjusted Event Rate of Adverse Events Leading to Study Drug Discontinuation in Either Treatment Phase Analysis Population: I/T/M (N = 285)

	Induction/Titration Phase N = 285 Treatment Duration Mean = 178 days; Median = 116 days; Range: 1 to 1607 days Total treatment exposure, person-years: 135.4		Maintenance Phase N = 223 Treatment Duration Mean = 739 days; Median = 697 days; Range: 5 to 1561 days Total treatment exposure, person-years: 444.1	
Adverse Events by SMQ/HLT/PT	Incidence [Number of Subjects (%)]	Exposure-Adjusted Event Rate [Number of Events/ Person-Year]	Incidence [Number of Subjects (%)]	Exposure-Adjusted Event Rate [Number of Events/ Person-Year]
Any AE leading to study drug discontinuation	32 (11%)	71 (0.52)	13 (6%)	21 (0.05)
Skin				
Injection site reaction (MedDRA high level term) [a]	4 (1%)	5 (0.04)	1 (<1%)	1 (<0.01)
Generalized skin reaction lasting ≥ 14 Days (Sponsor defined) [b]	5 (2%)	6 (0.04)	1 (<1%)	1 (<0.01)
Pruritus	3 (1%)	3 (0.02)	0 (0%)	0 (0)
Hyperhidrosis	1 (<1%)	1 (0.01)	0 (0%)	0 (0)
Pruritus generalised	1 (<1%)	2 (0.01)	0 (0%)	0 (0)
Musculoskeletal				
Arthralgia (Sponsor defined) [c]	9 (3%)	9 (0.07)	1 (<1%)	2 (<0.01)
Immunologic				
Hypersensitivity [d]	14 (5%)	24 (0.18)	6 (3%)	6 (0.01)
Neurologic				
Dizziness	2 (1%)	2 (0.01)	0 (0%)	0 (0)
Hypoaesthesia	1 (<1%)	1 (0.01)	0 (0%)	0 (0)
Presyncope	1 (<1%)	1 (0.01)	0 (0%)	0 (0)
Unresponsive to stimuli	1 (<1%)	1 (0.01)	0 (0%)	0 (0)
Paraesthesia	0 (0%)	0 (0)	1 (<1%)	1 (<0.01)
Gastrointestinal				
Diarrhea	1 (<1%)	1 (0.01)	0 (0%)	0 (0)
Vomiting	1 (<1%)	1 (0.01)	0 (0%)	0 (0)
Nausea	0 (0%)	0 (0)	1 (<1%)	2 (<0.01)
Respiratory				
Dysphonia	1 (<1%)	1 (0.01)	0 (0%)	0 (0)
General				
Contusion	1 (<1%)	2 (0.01)	0 (0%)	0 (0)
Chills	1 (<1%)	1 (0.01)	0 (0%)	0 (0)
Electrocution	1 (<1%)	1 (0.01)	0 (0%)	0 (0)
Malaise	1 (<1%)	1 (0.01)	0 (0%)	0 (0)
Oedema peripheral	1 (<1%)	1 (0.01)	0 (0%)	0 (0)
Pyrexia	1 (<1%)	1 (0.01)	0 (0%)	0 (0)
Psychiatric				
Anxiety	0 (0%)	0 (0)	1 (<1%)	1 (<0.01)

Brief psychotic disorder with marked stressors	0 (0%)	0 (0)	1 (<1%)	1 (<0.01)
Parasomnia	0 (0%)	0 (0)	1 (<1%)	1 (<0.01)
Urinary				
Urinary tract infection	1 (<1%)	1 (0.01)	0 (0%)	0 (0)
Renal				
Proteinuria	0 (0%)	0 (0)	1 (<1%)	1 (<0.01)
Blood and lymphatic				
Lymphoid tissue hyperplasia	1 (<1%)	1 (0.01)	0 (0%)	0 (0)
Infections				
Soft tissue infection	1 (<1%)	1 (0.01)	0 (0%)	0 (0)
Investigations				
C-reactive protein abnormal	1 (<1%)	1 (0.01)	0 (0%)	0 (0)
C-reactive protein increased	1 (<1%)	1 (0.01)	0 (0%)	0 (0)
Complement factor C4 decreased	1 (<1%)	1 (0.01)	0 (0%)	0 (0)
Alanine aminotransferase increased	0 (0%)	0 (0)	1 (<1%)	1 (<0.01)
Aspartate aminotransferase increased	0 (0%)	0 (0)	1 (<1%)	1 (<0.01)
Gamma-glutamyltransferase increased	0 (0%)	0 (0)	1 (<1%)	1 (<0.01)
Urine albumin/creatinine ratio increased	0 (0%)	0 (0)	1 (<1%)	1 (<0.01)

AESI, adverse event of special interest; FDA, Food and Drug Administration; HLT, high-level term; I/T/M, Induction/Titration/Maintenance; MedDRA, Medical Dictionary for Regulatory Activities; SMQ, Standardized MedDRA Query; PT, preferred term.

Treatment duration is defined as the duration from the first dose administered to last dose administered within each treatment phase.

Exposure-adjusted event rate used the exposure duration (person-years) from first dose administered to last dose administered within each treatment phase. Intervals of missing doses that were > 28 days were excluded. Maintenance Phase was defined as when subjects reached stable dose for 8 weeks. AEs for subjects receiving placebo during Maintenance Phase included.

[a] Terms reported include: injection site reaction and injection site mass. Derived using MedDRA high level term.

[b] Terms reported include: macule, pruritus, rash generalised, urticaria, and xanthogranuloma.

[c] Terms reported include: arthralgia and back pain.

[d] Terms reported include: anaphylaxis (per FDA adjudication), cough, hypersensitivity, hyperventilation, lip edema, lip swelling, local swelling, edema mouth, rash, rash generalised, serum sickness, swelling face, swollen tongue, tongue edema, urticaria, bronchospasm. Derived using hypersensitivity modified narrow SMQ with FDA adjudicated Anaphylaxis; injection site rash, injection site urticaria, anaphylactic reaction, and anaphylactoid reaction were excluded from the hypersensitivity narrow SMQ.

Source: **Table 15.1 (M1.11.3 SN0056); Table 16.1.1; Table 16.2.1; Listing 16.2.1.1**

Source: Applicant's submission, dated 4/10/2018, Module 1.11.3 Response to Request for Clinical Information Dated 26Mar2018, Table 3: Incidence and Exposure-Adjusted Event Rate of Adverse Events Leading to Study Drug Discontinuation in Either Treatment Phase Analysis Population: I/T/M (N = 285), pages 8 – 11.

Exposure-adjusted event rates of AEs leading to study drug discontinuation were similar across doses during the Maintenance period.

Table 37: Incidence and Exposure-Adjusted Event Rate of AEs Leading to Study Drug Discontinuation in the Maintenance Period

Table 4: Incidence and Exposure-Adjusted Event Rate of Adverse Events Leading to Study Drug Discontinuation in the Maintenance Phase (N = 223)

Adverse Events by SMQ/HLT/PT	Maintenance Phase (N = 223)					
	Dose on or Prior to Time of Adverse Event Onset					
Number of Subjects with Event (%) Number of Events (event rate/person-year)	Placebo (N = 28)	> 0 and < 20 mg/day (N = 52)	20 mg/day[a] (N = 164)	40 mg/day[b] (N = 197)	60 mg/day (N = 82)	Any Dose Level (N = 223)
Total treatment exposure, person-years	4.2	46.2	104.3	234.7	54.6	444.1
Total exposure, days						
Mean (SD)	55 (10.9)	325 (372.4)	232 (217.3)	435 (303.7)	243 (276.2)	727 (372.5)
Median	56	190	195	408	165	690
Min, Max	11 , 64	1 , 1512	1 , 1128	2 , 1185	1 , 875	5 , 1561
Any AE leading to study drug discontinuation	0	1 (1.9%) 3 (0.06)	3 (1.8%) 5 (0.05)	8 (4.1%) 10 (0.04)	1 (1.2%) 3 (0.05)	13 (5.8%) 21 (0.05)
Skin						
Injection site reaction (MedDRA high level term)[c]	0	1 (1.9%) 1 (0.02)	0	0	0	1 (0.4%) 1 (<0.01)
Generalized skin reaction lasting ≥ 14 Days (Sponsor defined)[d]	0	0	0	1 (0.5%) 1 (<0.01)	0	1 (0.4%) 1 (<0.01)
Musculoskeletal						
Arthralgia (Sponsor defined)[e]	0	0	0	1 (0.5%) 2 (0.01)	0	1 (0.4%) 2 (<0.01)
Immunologic						
Hypersensitivity[f]	0	0	1 (0.6%) 1 (0.01)	5 (2.5%) 5 (0.02)	0	6 (2.7%) 6 (0.01)
Neurologic						
Paraesthesia	0	1 (1.9%) 1 (0.02)	0	0	0	1 (<1%) 1 (<0.01)
Gastrointestinal						
Nausea	0	0	0	1 (0.5%) 2 (0.01)	0	1 (<1%) 2 (<0.01)
Psychiatric						
Anxiety	0	1 (1.9%) 1 (0.02)	0	0	0	1 (<1%) 1 (<0.01)
Brief psychotic disorder with marked stressors	0	0	1 (0.6%) 1 (0.01)	0	0	1 (<1%) 1 (<0.01)
Parasomnia	0	0	1 (0.6%) 1 (0.01)	0	0	1 (<1%) 1 (<0.01)
Renal						
Proteinuria	0	0	1 (0.6%) 1 (0.01)	0	0	1 (<1%) 1 (<0.01)
Investigations						
Alanine aminotransferase increased	0	0	0	0	1 (1.2%) 1 (0.02)	1 (<1%) 1 (<0.01)
Aspartate aminotransferase increased	0	0	0	0	1 (1.2%) 1 (0.02)	1 (<1%) 1 (<0.01)
Gamma-glutamyltransferase increased	0	0	0	0	1 (1.2%) 1 (0.02)	1 (<1%) 1 (<0.01)
Urine albumin/creatinine ratio increased	0	0	1 (0.6%) 1 (0.01)	0	0	1 (<1%) 1 (<0.01)

AESI, adverse event of special interest; FDA, Food and Drug Administration; HLT, high-level term; I/T/M, Induction/Titration/Maintenance; MedDRA, Medical Dictionary for Regulatory Activities; SMQ, Standardized MedDRA Query; PT, preferred term.

Total exposure (days) and total treatment exposure (person-years) used the aggregated duration (person-year) of treatment across all subjects with each dose level. Intervals of missing doses that were > 28 days were excluded. Maintenance Phase was defined as when subjects reached stable dose for 8 weeks. AEs for subjects receiving placebo during Maintenance Phase included.

[a] Exposure duration on 20 mg/day dose included ≥ 20 mg/day to < 40 mg/day doses.

[b] Exposure duration on 40 mg/day dose included ≥ 40 mg/day to < 60 mg/day doses.

[c] Terms reported include: injection site reaction. Derived using MedDRA high level term.

[d] Terms reported include: xanthogranuloma.

[e] Terms reported include: arthralgia and back pain.

[f] Terms reported include: anaphylaxis (per FDA adjudication), swollen tongue, tongue edema. Derived using hypersensitivity modified narrow SMQ with FDA adjudicated Anaphylaxis; injection site rash, injection site urticaria, anaphylactic reaction, and anaphylactoid reaction were excluded from the hypersensitivity narrow SMQ.

Source: Table 15.2 (M1.11.3 SN0056); Table 16.1.2; Table 16.2.2; Listing 16.2.1.1

Source: Applicant's submission, dated 4/10/2018, Module 1.11.3 Response to Request for Clinical Information Dated 26Mar2018, Table 4: Incidence and Exposure-Adjusted Event Rate of Adverse Events Leading to Study Drug Discontinuation in the Maintenance Phase (N = 223), pages 12 – 14.

Treatment Emergent Adverse Events (TEAEs)

The tables below show the incidence and exposure-adjusted event rates of TEAEs by treatment period with ≥ 10% incidence rate in either treatment period. For the I/T/M subjects who reached the maintenance period, the AEs are broken down by dose on or prior to AE event onset in the maintenance period to allow for comparison of dose response. There is no control data to compare AE rates, as all subjects were exposed to pegvaliase during the trials. The placebo subjects in the maintenance table are those subjects who reached the maintenance period with pegvaliase treatment and were then administered placebo during Part 2 of 165-302 in the randomized withdrawal trial. So, AEs that occur in high frequency in the general population and possibly not drug related, such as headache, nausea, nasopharyngitis, and fatigue, are included in these tables.

The AEs with the highest incidence were immune mediated or related to drug administration. These include injection site reactions, arthralgia, and hypersensitivity reactions. The incidence of these AEs was over 50% of subjects in both the induction/titration and maintenance periods. In addition, the exposure-adjusted event rate was at least three times greater in the induction/titration period compared to the maintenance period for injection site reactions, arthralgia, and hypersensitivity reactions. This suggests that these AEs correlate with the early immune response when ADA and CIC increased and peaked, and complements C3 and C4 dropped to the lowest levels.

The exposure-adjusted event rate was generally higher or approximately equal in the induction/titration period compared to the maintenance period for the non-immune mediated AEs. This includes the other organ systems, such as neurologic, gastrointestinal and respiratory. Of note, the psychiatric AEs of anxiety and depression were relatively constant between the two periods. These AEs are likely multifactorial, but potentially could have improved with the benefits of the drug on subjects' lifestyles or well-being, or worsened with the risks of immunologic complications.

Evaluating the exposure-adjusted event rates for hypersensitivity reactions showed a higher rate of 1.7 in the 40 mg/day group compared to a rate of 1.2 in the 20 mg/day group. This trend was not seen with injection site reactions or arthralgia which were higher in the 20 mg/day group compared to the 40 mg/day group. Generalized skin reactions lasting at least 14 days had approximately equal exposure-adjusted event rates between the groups. The other AEs demonstrate similar rates across dose levels, including placebo, during maintenance with no apparent trend. None of the generalized skin reactions was associated with mucosal involvement.

Table 38: Incidence and Exposure-Adjusted Event Rate of TEAEs by Treatment Phase (≥ 10% Incidence in Either Treatment Phase- I/T/M population)

	Induction/Titration Phase N = 285 Treatment Duration Mean = 178 day; Median = 116 days; Range: 1 to 1607 days Total treatment exposure, person-years: 135.4		**Maintenance Phase N = 223** Treatment Duration Mean = 739 day; Median = 697 days; Range: 5 to 1561 days Total treatment exposure, person-years: 444.1	
	Incidence [Number of Subjects (%)]	**Exposure-Adjusted Event Rate (Number of Events/Person-Years)**	**Incidence [Number of Subjects (%)]**	**Exposure-Adjusted Event Rate (Number of Events/Person-Years)**
Adverse Events by SMQ/HLT/PT				
Skin				
Injection site reaction (MedDRA high level term)	252 (88%)	2964 (21.89)	161 (72%)	1754 (3.95)
Injection site reaction (MedDRA High Level Term) lasting ≥ 14 Days	68 (24%)	138 (1.02)	67 (30%)	190 (0.43)
Generalized skin reaction lasting ≥ 14 Days (Sponsor defined)	61 (21%)	95 (0.70)	82 (37%)	133 (0.30)
Pruritus	58 (20%)	100 (0.74)	53 (24%)	402 (0.91)
Alopecia	13 (5%)	14 (0.10)	39 (17%)	50 (0.11)
Musculoskeletal				
Arthralgia[a]	210 (74%)	1035 (7.64)	137 (61%)	661 (1.49)
Myalgia	23 (8%)	33 (0.24)	25 (11%)	38 (0.09)
Immunologic				
Hypersensitivity[b]	152 (53%)	633 (4.68)	135 (61%)	663 (1.49)

Neurologic				
Headache[c]	100 (35%)	211 (1.56)	111 (50%)	778 (1.75)
Dizziness	46 (16%)	64 (0.47)	38 (17%)	72 (0.16)
Gastrointestinal				
Nausea	51 (18%)	66 (0.49)	57 (26%)	106 (0.24)
Abdominal pain[d]	39 (14%)	53 (0.39)	55 (25%)	128 (0.29)
Vomiting	36 (13%)	53 (0.39)	58 (26%)	100 (0.23)
Diarrhoea	25 (9%)	31 (0.23)	50 (22%)	91 (0.20)
Oropharyngeal pain	38 (13%)	43 (0.32)	51 (23%)	70 (0.16)
Dyspepsia	15 (5%)	18 (0.13)	26 (12%)	68 (0.15)
Respiratory				
Nasopharyngitis	47 (16%)	60 (0.44)	89 (40%)	206 (0.46)
Upper respiratory tract infection	41 (14%)	79 (0.58)	85 (38%)	180 (0.41)
Cough	27 (9%)	33 (0.24)	50 (22%)	65 (0.15)
Sinusitis[e]	27 (9%)	30 (0.22)	52 (23%)	103 (0.23)
Nasal congestion	12 (4%)	15 (0.11)	41 (18%)	50 (0.11)
Bronchitis	4 (1%)	5 (0.04)	26 (12%)	35 (0.08)
General				
Fatigue	37 (13%)	81 (0.60)	48 (22%)	86 (0.19)
Pyrexia	36 (13%)	65 (0.48)	29 (13%)	38 (0.09)
Pain	31 (11%)	50 (0.37)	25 (11%)	36 (0.08)
Contusion	29 (10%)	44 (0.32)	37 (17%)	63 (0.14)
Erythema	22 (8%)	39 (0.29)	23 (10%)	43 (0.10)
Insomnia	8 (3%)	9 (0.07)	26 (12%)	37 (0.08)
Seasonal allergy	7 (2%)	12 (0.09)	32 (14%)	41 (0.09)
Psychiatric				
Anxiety	14 (5%)	23 (0.17)	41 (18%)	79 (0.18)
Depression	3 (1%)	3 (0.02)	27 (12%)	34 (0.08)
Urinary				
Urinary tract infection	10 (4%)	13 (0.10)	26 (12%)	31 (0.07)

FDA, Food and Drug Administration; HLT, high-level term; MedDRA, Medical Dictionary for Regulatory Activities; SMQ, Standardized MedDRA Query; PT, preferred term.
Exposure-adjusted event rate used the duration (person-years) from first dose administered to last dose administered within each treatment phase. Maintenance Phase was defined as when subjects reached stable dose for 8 weeks. AEs for subjects receiving placebo during Maintenance Phase included

[a] Arthralgia combines preferred terms of arthralgia, back pain, musculoskeletal pain, pain in extremity, and neck pain.

[b] Hypersensitivity modified narrow SMQ with FDA adjudicated Anaphylaxis. Injection site rash, injection site urticaria, anaphylactic reaction, and anaphylactoid reaction were excluded from the hypersensitivity narrow SMQ.

[c] Headache combines headache, migraine, and sinus headache.

[d] Abdominal pain combines abdominal pain, abdominal pain upper, and abdominal discomfort.

[e] Sinusitis combines sinusitis and sinus congestion.

Source: Table 15.1, Table 15.3, and Table 15.19.

Source: Applicant's submission, dated 3/19/18, Module 1.11.3 Clinical Information Amendment, Response to Clinical Information Request dated 07Mar2018, Table 1: Incidence and Exposure-Adjusted Event Rate of Adverse Events by Treatment Phase With ≥ 10% Incidence Rate in Either Treatment Phase, pages 2-4.

Table 39: Incidence and Exposure-Adjusted Event Rate of TEAEs (≥ 10% of Subjects) during the Maintenance Phase by Actual Dose at Event Onset (N = 223)

	Maintenance Phase (N = 223)					
	Dose on or Prior to Time of Adverse Event Onset					
Number of Subjects with Event (%) **Number of Events (event rate/person-year)**	**Placebo (N = 28)**	**> 0 and < 20 mg/day (N = 52)**	**20 mg/day (N = 164)**	**40 mg/day (N = 197)**	**60 mg/day (N = 82)**	**Any Dose Level (N = 223)**
Total treatment exposure, person-years	4.2	46.2	104.3	234.7	54.6	444.1
Total exposure, days						
Mean (SD)	55 (10.9)	325 (372.4)	232 (217.3)	435 (303.7)	243 (276.2)	727 (372.5)
Median	56	190	195	408	165	690
Min, Max	11 , 64	1 , 1512	1 , 1128	2 , 1185	1 , 875	5 , 1561
Skin						
Injection site reaction (MedDRA high level term)	7 (25.0%) 10 (2.35)	10 (19.2%) 191 (4.13)	65 (39.6%) 710 (6.81)	120 (60.9%) 718 (3.06)	24 (29.3%) 125 (2.29)	161 (72.2%) 1754 (3.95)
Injection site reaction (MedDRA High Level Term) lasting ≥ 14 Days	1 (3.6%) 1 (0.24)	3 (5.8%) 44 (0.95)	20 (12.2%) 50 (0.48)	46 (23.4%) 73 (0.31)	12 (14.6%) 22 (0.40)	67 (30.0%) 190 (0.43)
Generalized skin reaction lasting ≥ 14 Days (Sponsor defined)	0 0	6 (11.5%) 10 (0.22)	23 (14.0%) 32 (0.31)	55 (27.9%) 79 (0.34)	10 (12.2%) 12 (0.22)	82 (36.8%) 133 (0.30)
Pruritus	1 (3.6%) 1 (0.24)	2 (3.8%) 30 (0.65)	17 (10.4%) 36 (0.35)	37 (18.8%) 312 (1.33)	11 (13.4%) 23 (0.42)	53 (23.8%) 402 (0.91)
Alopecia	3 (10.7%) 3 (0.71)	4 (7.7%) 4 (0.09)	16 (9.8%) 18 (0.17)	20 (10.2%) 21 (0.09)	4 (4.9%) 4 (0.07)	39 (17.5%) 50 (0.11)
Musculoskeletal						
Arthralgia[a]	5 (17.9%) 6 (1.41)	18 (34.6%) 56 (1.21)	55 (33.5%) 210 (2.01)	93 (47.2%) 333 (1.42)	23 (28.0%) 56 (1.03)	137 (61.4%) 661 (1.49)
Myalgia	1 (3.6%) 1 (0.24)	2 (3.8%) 2 (0.04)	5 (3.0%) 10 (0.10)	19 (9.6%) 24 (0.10)	1 (1.2%) 1 (0.02)	25 (11.2%) 38 (0.09)
Immunologic						
Hypersensitivity[b]	1 (3.6%) 1 (0.24)	15 (28.8%) 76 (1.64)	50 (30.5%) 128 (1.23)	102 (51.8%) 403 (1.72)	17 (20.7%) 55 (1.01)	135 (60.5%) 663 (1.49)
Neurologic						
Headache[c]	8 (28.6%) 18 (4.24)	12 (23.1%) 148 (3.20)	49 (29.9%) 174 (1.67)	74 (37.6%) 315 (1.34)	21 (25.6%) 123 (2.25)	111 (49.8%) 778 (1.75)
Dizziness	1 (3.6%) 2 (0.47)	7 (13.5%) 12 (0.26)	10 (6.1%) 11 (0.11)	19 (9.6%) 36 (0.15)	8 (9.8%) 11 (0.20)	38 (17.0%) 72 (0.16)
Gastrointestinal						
Nausea	2 (7.1%) 3 (0.71)	7 (13.5%) 10 (0.22)	19 (11.6%) 28 (0.27)	29 (14.7%) 48 (0.20)	13 (15.9%) 17 (0.31)	57 (25.6%) 106 (0.24)
Abdominal pain[d]	1 (3.6%)	7 (13.5%)	16 (9.8%)	29 (14.7%)	8 (9.8%)	55 (24.7%)

	1 (0.24)	11 (0.24)	30 (0.29)	76 (0.32)	10 (0.18)	128 (0.29)
Vomiting	1 (3.6%) 1 (0.24)	4 (7.7%) 6 (0.13)	19 (11.6%) 27 (0.26)	36 (18.3%) 51 (0.22)	12 (14.6%) 15 (0.27)	58 (26.0%) 100 (0.23)
Diarrhoea	0	6 (11.5%) 7 (0.15)	17 (10.4%) 21 (0.20)	30 (15.2%) 48 (0.20)	9 (11.0%) 15 (0.27)	50 (22.4%) 91 (0.20)
Oropharyngeal pain	1 (3.6%) 1 (0.24)	2 (3.8%) 2 (0.04)	15 (9.1%) 18 (0.17)	32 (16.2%) 45 (0.19)	4 (4.9%) 4 (0.07)	51 (22.9%) 70 (0.16)
Dyspepsia	0	1 (1.9%) 1 (0.02)	13 (7.9%) 18 (0.17)	15 (7.6%) 30 (0.13)	3 (3.7%) 19 (0.35)	26 (11.7%) 68 (0.15)
Respiratory						
Nasopharyngitis	1 (3.6%) 1 (0.24)	10 (19.2%) 17 (0.37)	33 (20.1%) 52 (0.50)	68 (34.5%) 121 (0.52)	9 (11.0%) 15 (0.27)	89 (39.9%) 206 (0.46)
Upper respiratory tract infection	5 (17.9%) 5 (1.18)	8 (15.4%) 13 (0.28)	35 (21.3%) 56 (0.54)	51 (25.9%) 94 (0.40)	10 (12.2%) 12 (0.22)	85 (38.1%) 180 (0.41)
Cough	2 (7.1%) 2 (0.47)	1 (1.9%) 2 (0.04)	22 (13.4%) 25 (0.24)	24 (12.2%) 28 (0.12)	8 (9.8%) 8 (0.15)	50 (22.4%) 65 (0.15)
Sinusitis[e]	0	9 (17.3%) 17 (0.37)	21 (12.8%) 26 (0.25)	27 (13.7%) 49 (0.21)	8 (9.8%) 11 (0.20)	52 (23.3%) 103 (0.23)
Nasal congestion	3 (10.7%) 4 (0.94)	4 (7.7%) 5 (0.11)	15 (9.1%) 15 (0.14)	20 (10.2%) 21 (0.09)	5 (6.1%) 5 (0.09)	41 (18.4%) 50 (0.11)
Bronchitis	2 (7.1%) 2 (0.47)	2 (3.8%) 2 (0.04)	9 (5.5%) 12 (0.12)	13 (6.6%) 16 (0.07)	2 (2.4%) 3 (0.05)	26 (11.7%) 35 (0.08)
General						
Fatigue	3 (10.7%) 3 (0.71)	3 (5.8%) 3 (0.06)	20 (12.2%) 29 (0.28)	25 (12.7%) 36 (0.15)	8 (9.8%) 15 (0.27)	48 (21.5%) 86 (0.19)
Pyrexia	0	4 (7.7%) 4 (0.09)	8 (4.9%) 9 (0.09)	14 (7.1%) 16 (0.07)	6 (7.3%) 9 (0.16)	29 (13.0%) 38 (0.09)
Pain	0	3 (5.8%) 4 (0.09)	6 (3.7%) 8 (0.08)	16 (8.1%) 20 (0.09)	4 (4.9%) 4 (0.07)	25 (11.2%) 36 (0.08)
Contusion	2 (7.1%) 6 (1.41)	6 (11.5%) 8 (0.17)	12 (7.3%) 16 (0.15)	21 (10.7%) 29 (0.12)	3 (3.7%) 4 (0.07)	37 (16.6%) 63 (0.14)
Erythema	0	1 (1.9%) 2 (0.04)	9 (5.5%) 15 (0.14)	14 (7.1%) 20 (0.09)	4 (4.9%) 6 (0.11)	23 (10.3%) 43 (0.10)
Insomnia	0	3 (5.8%) 3 (0.06)	6 (3.7%) 8 (0.08)	18 (9.1%) 23 (0.10)	3 (3.7%) 3 (0.05)	26 (11.7%) 37 (0.08)
Seasonal allergy	0	4 (7.7%) 6 (0.13)	10 (6.1%) 13 (0.12)	18 (9.1%) 20 (0.09)	2 (2.4%) 2 (0.04)	32 (14.3%) 41 (0.09)
Psychiatric						
Anxiety	2 (7.1%) 2 (0.47)	5 (9.6%) 5 (0.11)	15 (9.1%) 21 (0.20)	27 (13.7%) 44 (0.19)	4 (4.9%) 7 (0.13)	41 (18.4%) 79 (0.18)
Depression	2 (7.1%) 2 (0.47)	5 (9.6%) 6 (0.13)	10 (6.1%) 10 (0.10)	12 (6.1%) 13 (0.06)	2 (2.4%) 3 (0.05)	27 (12.1%) 34 (0.08)
Urinary						
Urinary tract infection	0	2 (3.8%) 2 (0.04)	7 (4.3%) 8 (0.08)	16 (8.1%) 18 (0.08)	3 (3.7%) 3 (0.05)	26 (11.7%) 31 (0.07)

FDA, Food and Drug Administration; HLT, high-level term; Max, maximum; MedDRA, Medical Dictionary for Regulatory Activities; Min, minimum; SD, standard deviation; SMQ, Standardized MedDRA Query; PT, preferred term.
Exposure-adjusted event rate used the duration (person-years) from first dose administered to last dose administered within each treatment phase. Maintenance Phase was defined as when subjects reached stable dose for 8 weeks. Any dose level includes AEs for subjects receiving placebo during Maintenance Phase.

[a] Arthralgia combines preferred terms of arthralgia, back pain, musculoskeletal pain, pain in extremity, and neck pain.

[b] Hypersensitivity modified narrow SMQ with FDA adjudicated Anaphylaxis. Injection site rash and injection site urticaria were excluded from the hypersensitivity narrow SMQ. Reported terms included

[c] Headache combines headache, migraine, and sinus headache.

[d] Abdominal pain combines abdominal pain, abdominal pain upper, and abdominal discomfort.

[e] Sinusitis combines sinusitis and sinus congestion.

Source: Table 15.2, Table 15.11, and Table 15.20.

Source: Applicant's submission, dated 3/19/18, Module 1.11.3 Clinical Information Amendment, Response to Clinical Information Request dated 07Mar2018, Table 2: Incidence and Exposure-Adjusted Event Rate of Adverse Events Occurring in at Least 10% of Subjects Treated with PALYNZIQ in the Maintenance Phase (N = 223), pages 5-9.

Table 40 shows preferred terms with an event rate ≥ 1 (event/person-years) in at least one individual treatment arm for subjects in Trial 165-302, Part 2. In the active groups, the event rates were 49.7 events/person-years for the 20 mg/day group and 34.9 events/person-years for the 40 mg/day group. The placebo groups had lower event rates, 30.5 events/person-years in the 20 mg/day group and 27.5 events/person-years in the 40 mg/day group. As expected, arthralgia event rates are higher in the active groups than the placebo groups. Notably, the arthralgia event rates are about 3 times higher in the 20 mg/day group compared to the 40 mg/day group. Event rates of complications with the injection site are generally higher in the active than placebo groups, although some are higher in the 20 mg/day active group and others are higher in the 40 mg/day active group. Event rates of AEs that are common in the general population, such as nausea, upper respiratory tract infection, and cough, are similar between the active and placebo groups. This data has the limitations that the treatment period is only 8 weeks and the sample size is small.

Table 40: Event Rate of AE ≥ 1 Event/Person-years by SOC and Preferred Terms, Trial 165-302, Part 2 Safety Population

	20mg/day Active (N=34)	40mg/day Active (N=32)	20mg/day Placebo (N=15)	40mg/day Placebo (N=14)	Pooled Active (N=66)	Pooled Placebo (N=29)
Exposure, person-years	5.2	4.6	2.3	2.1	9.8	4.4
Total Number of Adverse Events (Event Rate)	259 (49.7)	161 (34.9)	70 (30.5)	57 (27.5)	420 (42.7)	127 (29.1)
General disorders and administration site conditions	**91 (17.4)**	**57 (12.4)**	**11 (4.8)**	**5 (2.4)**	**148 (15.1)**	**16 (3.7)**
Injection site pain	4 (0.8)	24 (5.2)	1 (0.4)	0 (0.0)	28 (2.8)	1 (0.2)
Injection site swelling	25 (4.8)	0 (0.0)	0 (0.0)	2 (1.0)	25 (2.5)	2 (0.5)
Injection site reaction	20 (3.8)	1 (0.2)	1 (0.4)	1 (0.5)	21 (2.1)	2 (0.5)

	20mg/day Active (N=34)	40mg/day Active (N=32)	20mg/day Placebo (N=15)	40mg/day Placebo (N=14)	Pooled Active (N=66)	Pooled Placebo (N=29)
Injection site erythema	10 (1.9)	8 (1.7)	0 (0.0)	0 (0.0)	18 (1.8)	0 (0.0)
Injection site haemorrhage	1 (0.2)	16 (3.5)	0 (0.0)	0 (0.0)	17 (1.7)	0 (0.0)
Fatigue	6 (1.2)	3 (0.7)	3 (1.3)	0 (0.0)	9 (0.9)	3 (0.7)
Injection site pruritus	7 (1.3)	1 (0.2)	0 (0.0)	0 (0.0)	8 (0.8)	0 (0.0)
Injection site bruising	4 (0.8)	1 (0.2)	4 (1.7)	1 (0.5)	5 (0.5)	5 (1.1)
Musculoskeletal and connective tissue disorders	**52 (10.0)**	**18 (3.9)**	**3 (1.3)**	**4 (1.9)**	**70 (7.1)**	**7 (1.6)**
Arthralgia	49 (9.4)	13 (2.8)	1 (0.4)	2 (1.0)	62 (6.3)	3 (0.7)
Nervous system disorders	**28 (5.4)**	**11 (2.4)**	**18 (7.8)**	**12 (5.8)**	**39 (4.0)**	**30 (6.9)**
Headache	15 (2.9)	2 (0.4)	4 (1.7)	9 (4.3)	17 (1.7)	13 (3.0)
Migraine	0 (0.0)	2 (0.4)	4 (1.7)	0 (0.0)	2 (0.2)	4 (0.9)
Psychiatric disorders	**12 (2.3)**	**26 (5.6)**	**6 (2.6)**	**5 (2.4)**	**38 (3.9)**	**11 (2.5)**
Anxiety	4 (0.8)	5 (1.1)	0 (0.0)	2 (1.0)	9 (0.9)	2 (0.5)
Agitation	0 (0.0)	6 (1.3)	0 (0.0)	0 (0.0)	6 (0.6)	0 (0.0)
Mood altered	0 (0.0)	1 (0.2)	0 (0.0)	2 (1.0)	1 (0.1)	2 (0.5)
Skin and subcutaneous tissue disorders	**18 (3.5)**	**14 (3.0)**	**3 (1.3)**	**2 (1.0)**	**32 (3.3)**	**5 (1.1)**
Pruritus	1 (0.2)	6 (1.3)	0 (0.0)	1 (0.5)	7 (0.7)	1 (0.2)
Urticaria	5 (1.0)	1 (0.2)	0 (0.0)	0 (0.0)	6 (0.6)	0 (0.0)
Gastrointestinal disorders	**16 (3.1)**	**10 (2.2)**	**4 (1.7)**	**6 (2.9)**	**26 (2.6)**	**10 (2.3)**
Abdominal pain	5 (1.0)	0 (0.0)	0 (0.0)	0 (0.0)	5 (0.5)	0 (0.0)
Nausea	4 (0.8)	1 (0.2)	1 (0.4)	2 (1.0)	5 (0.5)	3 (0.7)
Infections and infestations	**17 (3.3)**	**7 (1.5)**	**6 (2.6)**	**7 (3.4)**	**24 (2.4)**	**13 (3.0)**
Upper respiratory tract infection	1 (0.2)	0 (0.0)	3 (1.3)	2 (1.0)	1 (0.1)	5 (1.1)
Reproductive system and breast disorders	**8 (1.5)**	**3 (0.7)**	**0 (0.0)**	**1 (0.5)**	**11 (1.1)**	**1 (0.2)**
Dysmenorrhoea	8 (1.5)	0 (0.0)	0 (0.0)	1 (0.5)	8 (0.8)	1 (0.2)
Investigations	**4 (0.8)**	**6 (1.3)**	**4 (1.7)**	**2 (1.0)**	**10 (1.0)**	**6 (1.4)**
Respiratory, thoracic and mediastinal disorders	**5 (1.0)**	**4 (0.9)**	**4 (1.7)**	**4 (1.9)**	**9 (0.9)**	**8 (1.8)**
Nasal congestion	1 (0.2)	0 (0.0)	4 (1.7)	0 (0.0)	1 (0.1)	4 (0.9)
Cough	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.0)	0 (0.0)	2 (0.5)
Immune system disorders	**2 (0.4)**	**2 (0.4)**	**0 (0.0)**	**0 (0.0)**	**4 (0.4)**	**0 (0.0)**
Injury, poisoning and procedural complications	**2 (0.4)**	**1 (0.2)**	**8 (3.5)**	**2 (1.0)**	**3 (0.3)**	**10 (2.3)**
Contusion	1 (0.2)	1 (0.2)	6 (2.6)	0 (0.0)	2 (0.2)	6 (1.4)
Surgical and medical procedures	**1 (0.2)**	**1 (0.2)**	**0 (0.0)**	**0 (0.0)**	**2 (0.2)**	**0 (0.0)**
Blood and lymphatic system disorders	**1 (0.2)**	**0 (0.0)**	**0 (0.0)**	**2 (1.0)**	**1 (0.1)**	**2 (0.5)**
Lymphadenopathy	1 (0.2)	0 (0.0)	0 (0.0)	2 (1.0)	1 (0.1)	2 (0.5)
Cardiac disorders	**1 (0.2)**	**0 (0.0)**	**0 (0.0)**	**0 (0.0)**	**1 (0.1)**	**0 (0.0)**
Ear and labyrinth disorders	**0 (0.0)**	**1 (0.2)**	**1 (0.4)**	**2 (1.0)**	**1 (0.1)**	**3 (0.7)**

	20mg/day Active (N=34)	40mg/day Active (N=32)	20mg/day Placebo (N=15)	40mg/day Placebo (N=14)	Pooled Active (N=66)	Pooled Placebo (N=29)
Metabolism and nutrition disorders	**1 (0.2)**	**0 (0.0)**	**0 (0.0)**	**1 (0.5)**	**1 (0.1)**	**1 (0.2)**
Eye disorders	**0 (0.0)**	**0 (0.0)**	**1 (0.4)**	**0 (0.0)**	**0 (0.0)**	**1 (0.2)**
Renal and urinary disorders	**0 (0.0)**	**0 (0.0)**	**1 (0.4)**	**2 (1.0)**	**0 (0.0)**	**3 (0.7)**

Source: Applicant's submission, dated 6/30/2017, Module 5.3.5.1 165-302 Study Report Body, Table 14.3.1.4.39 Summary of Event Rate of Adverse Event by SOC and Preferred Terms. Analysis Population: Part 2 Safety Population 165-302 Part 2 Data, pages 4122 – 4138.

Laboratory Findings

Laboratory findings included data from reported AEs and analysis of laboratory data. As reported by the Sponsor in the IR response received 3/19/2018, "A clinical laboratory abnormality was first confirmed as a laboratory abnormality based on repeat laboratory measurement and then documented as an AE if considered clinically significant by the investigator. Clinical judgement, not specific cut-off values, were used for determining clinical significance of laboratories. Potential factors considered when evaluating clinical significance were as follows:

- Was accompanied by clinical symptoms
- Required a change in concomitant therapy (e.g., addition of; interruption of; discontinuation of; or any other change in a concomitant medication, therapy, or treatment).
- Suggested an abnormality related to a disease and/or organ toxicity
- The abnormality was of a degree that required active management (e.g., change of dose, discontinuation of study drug, more frequent follow-up assessments, further diagnostic investigation)"

The team felt that laboratory abnormalities determined to be AEs should only be based on objective criteria outside the normal range rather than clinical judgement. During labeling negotiations, the Sponsor was asked to reassess the data based on objective criteria for "increased" or "decreased" laboratory findings. The team felt that all abnormal laboratory findings should be included for review as causality cannot be convincingly attributed in the absence of a control arm. The renal abnormalities of increased urine albumin: creatinine ratio (UACR), proteinuria, hematuria, and increased serum creatinine were of concern for possible renal toxicity. This reviewer assessed the narratives and concluded that many of the abnormalities were present at baseline, transient, not progressive, or noted in subjects with risk factors for renal impairment, such as obesity and diabetes. This was reassuring that there was not a renal safety signal to date. The Sponsor consulted external nephrology experts to review data on subjects with ≥ 3 consecutive UACR ≥ 3 mg/mmol from the PAL-003 and 165-302

studies. "Based on the data that had been previously shared and reviewed, the external nephrology experts stated that they would not consider the microalbuminuria observed with pegvaliase treatment concerning or specific for immune complex glomerulonephritis" (Summary of Clinical Safety).

The review team consulted the Division of Cardiovascular and Renal Products for their nephrology expertise on whether there is evidence of drug-induced renal toxicity based on the submitted data. The consultant team reviewed all relevant patient narratives and laboratory data and concluded that "no subject exhibited a marked change in renal function or developed progressive or persistent proteinuria, and none of the narratives suggest an immune complex-mediated glomerulonephritis. One subject identified in the 120-day safety update was diagnosed with IgA nephropathy, but the diagnosis most likely pre-dated pegvaliase exposure given the subject's history of proteinuria and hematuria." The nephrology consultants concluded that "there is no signal for acute or chronic nephrotoxicity with pegvaliase based on the available clinical data." Refer to consult review by Dr. Kimberly Smith in DARRTS, dated 2/28/2018, for further details.

Reductions in C3 and C4 were noted. These trends and associated safety risk are discussed in the immunogenicity section.

CRP > ULN as well as hs-CRP concentration >0.287 mg/dL over a 6-month period were both elevated during the induction/titration and maintenance period of treatment and both showed a trend towards improvement over time possibly due to developing immune tolerance to the product. The Applicant proposed that this CRP elevation could be due to factors other than pegvaliase treatment, such as elevated BMI. However, patients with reported increases in CRP or hs-CRP largely did not exhibit those elevations at baseline and, thus, it is unlikely that baseline weight (which did not significantly change during the trials) would account for these laboratory abnormalities. Given the immunogenicity and high rate of immune-mediated adverse reactions in the pegvaliase trials, it is likely that elevations of CRP and hs-CRP, both markers of an inflammatory response, from baseline are due to pegvaliase treatment. However, in the absence of a control arm or further information on baseline CRP levels or changes in those over time and with diet in the PKU population, it is difficult to ascribe causality with confidence. As such, the inflammatory responses during pegvaliase treatment (including CRP and hs-CRP concentrations) will be further evaluated with chronic pegvaliase administration in the post-marketing setting.

In the Phase 3 program, there were no Hy's law cases of drug induced liver injury identified.

Hypophenylalanimia (blood Phe concentration < 30 micromol/L) on a single measurement was reported frequently with an exposure-adjusted event rate which increased over time as shown in Table 41 below. When encountered on a single measurement only as the rate increased over time, hypophenylalaninemia may be due to overtitrating to a target dose of 40 mg once daily as

was dictated by the protocol in Trial 165-302 Part 4. However, the rate of sustained hypophenylalaninemia (blood Phe concentration < 30 micromol/L on 2 or more consecutive measurements) was less frequent and remained relatively constant with longer duration of pegvaliase treatment, which may be due to dietary protein changes as dictated by the trial protocol for blood Phe concentration ≤ 30 micromol/L and/or to pegvaliase dose reduction. The product label includes instructions on dietary modification and/or dose reduction in the event of hypophenylalaninemia. In addition, the dosage instructions in the label recommend using the lowest effective dose of pegvaliase to achieve the desired therapeutic response (defined as ≥20% blood Phe reduction or blood Phe ≤ 600 micromol/L) and to increase the dose to 40 mg once daily (maximum) only when the lower dose (20 mg) has been maintained for at least 24 weeks to allow sufficient time to achieve a response.

Instances of blood CPK elevations were stable over time. For a discussion of this laboratory abnormality, refer to section 8.2.4 SAEs. Further evaluation of increased blood CPK will occur through the post-approval studies.

The types and rates of laboratory abnormalities (adjusted for duration of exposure) reported during the maintenance phase in patients receiving 20 mg once daily and 40 mg once daily were similar with the exception of hs-CRP above 0.287 mg/dL over a 6-month period (exposure-adjusted event rates 0.04 and 0.08 in patients on 20 mg once daily and 40 mg once daily respectively).

Table 41: Laboratory Abnormalities Occurring in at least 1% of Subjects Treated with pegvaliase by Treatment Period (I/T/M population)

Treatment Phase Treatment Duration	**Induction/Titration Phase (N = 285)** 135 person-years Mean: 178 days Median: 116 days Range: 1 to 1607 days		**Maintenance Phase (N = 223)** 444 person-years Mean: 739 days Median: 697 days Range: 5 to 1561 days	
Laboratory Measurement	**N (%)***	**Episodes (Rate)***	**N (%)***	**Episodes (Rate)***
Complement factor C3 < LLN	195 (68%)	446 (3.3)	188 (84%)	1719 (3.9)
C-reactive protein (CRP) > ULN	182 (64%)	358 (2.6)	151 (68%)	947 (2.1)
Complement factor C4 < LLN	177 (62%)	318 (2.4)	108 (48%)	604 (1.4)
Hypophenylalaninemia (blood phenylalanine concentration < 30 micromol/L) as a single measurement	53 (19%)	204 (1.5)	137 (61 %)	1128 (2.5)
Blood creatine phosphokinase (CPK) > ULN	50 (18%)	87 (0.6)	96 (43%)	277 (0.6)
Hypophenylalaninemia (blood phenylalanine concentration < 30 micromol/L) on 2 or more consecutive measurements	45 (16%)	60 (0.4)	93 (42%)	140 (0.3)
Hs-CRP > 0.287 mg/dL over a 6 month period	34 (12%)	34 (0.4)	23 (10%)	26 (0.06)
UACR ≥ 3 mg/mmol on 3 or more consecutive measurements	9 (3%)	9 (0.07)	6 (3%)	6 (0.01)
Hematuria > 3 RBC/hpf OR > ULN on 3 or more consecutive measurements	4 (1%)	10 (0.07)	7 (3%)	7 (0.02)
Serum creatinine > 30% above baseline OR > ULN on 3 or more consecutive measurements	2 (1%)	2 (0.01)	7 (3%)	7 (0.02)

* N (%) = Number of patients with at least 1 laboratory abnormality (%); Rate = Exposure-Adjusted Rate of Laboratory Abnormalities (Laboratory Abnormality/Person-Years)
LLN – lower limit of normal
ULN – upper limit of normal
Hs – high sensitivity
UACR – urinary albumin-creatinine ratio
RBC – red blood cell
Hpf – high power field

Source: Applicant's submission, dated 5/7/2018, Module 1.14.1.3 Draft prescribing information clean PDF, Table 3: Laboratory Abnormalities Occurring in at least 1% of PKU Patients Treated with Palynziq in an Induction/Titration/Maintenance Regimen in Clinical Trials – Incidence and Exposure-Adjusted Rates, page 10.

Vital Signs

Vital signs included systolic and diastolic blood pressure (mm Hg), heart rate (bpm), respiration rate (breaths per minute), and temperature (degrees Celsius). As noted in the Trial 165-301 CSR, "Changes in vital signs that were assessed as clinically significant by the investigator were to be reported as AEs; no vital sign changes met this criterion" (page 276). As noted in the 302 CSR, "Vital sign-related changes reported as AEs (by PT) included Pyrexia (18 events in 13 [6.0%] subjects), Hypertension (5 events in 5 [2.3%] subjects), Heart Rate Increased (6 events in 3 [1.4%] subjects), Tachycardia (3 events in 3 [1.4%] subjects), Blood Pressure Increased (2 events in 1 [0.5%] subject), Hypotension (1 event in 1 [0.5%] subject), and Secondary Hypertension (1 event in 1 [0.5%] subject" (page 422). There was one event of tachycardia which was an SAE and discussed below. Otherwise, all events were non-serious and Grade 1 or Grade 2 in severity. Since many of these events lack narratives, it is difficult to make an independent determination. However, it is reassuring to know that "most events had resolved or were resolving as of the data cutoff for this report, and 32 of the 36 AEs related to vital signs findings were assessed by the investigator as unrelated to study drug" (page 422). There was no change in pegvaliase dosing in response to these vital sign abnormalities, except for one event of Grade 2 pyrexia which led to treatment interruption and one event of Grade 2 heart rate increased which led to a dose reduction. Pyrexia and hypertension, for example, are relatively common in the adult population and without having full details of pre-existing conditions, concurrent illnesses, and other medications, causality is hard to establish. Given the low grade and non-serious nature of these events, vital sign abnormalities do not present a safety signal.

The narrative for the SAE of tachycardia (subject (b) (6)) was reviewed. This subject's medical history included left ventricular hypertrophy, hypertension, and anxiety. During study 301, he had an abnormal ECG and was referred for a cardiology workup. The results of the cardiology workup were not reported. When the subject was in Part 4 of Study 302, he experienced an episode of nausea, vomiting, and a "racing" heart. The subject was evaluated in the ED and had a normal workup. It is unclear what instigated the tachycardia episode, but the event did not recur and the subject continued treatment.

Electrocardiograms (ECGs)

A standard 12-lead ECG was to be recorded at baseline and after completion of study drug. Clinically significant abnormalities were determined by investigators. As discussed in the 301 CSR, there were 6 subjects with clinically significant abnormal ECGs at screening, but five of these subjects did not have clinically significant abnormal ECGs at study end. Subject (b) (6) had clinically significant abnormal ECGs at screening and study completion. The narrative for this subject was reviewed. This is the same subject discussed above with the tachycardia SAE. This subject's medical history included left ventricular hypertrophy, hypertension, and anxiety. Per the ECG Results Listing 16.2.8.2.3 provided with the Application's submission and

the 301 CSR, the study completion ECG reported "R-wave progression, early transition, probably left atrial enlargement and right ventricular hypertrophy. These findings were reported as a grade 1 AE assessed by the investigator as probably related to study drug" (page 277). These ECG findings are concerning, but the subject also had pre-existing cardiac history which makes causality difficult to determine.

3 additional subjects had clinically significant abnormal ECGs at study 301 end. As reported in Listing 16.2.8.2.3 and the 301 CSR, two subjects had sinus bradycardia at the end of study visit, one reported as grade 2 and one reported as grade 1. Both subjects have a history of hypertension. The third subject ((b) (6)) had borderline right axis deviation reported as a grade 1 AE assessed by the investigator as not related to study drug. A review of this subject's medical history does not reveal any cardiac history. There were no narratives for these subjects that included discussion of these ECG findings.

As discussed in the 302 CSR, "Of the 16 subjects in 165-302 with post-baseline ECGs, 1 (6.3%) had a reported clinically significant abnormality of sinus bradycardia. All other ECGs were either interpreted as normal or as abnormal but not clinically significant" (page 423). There was no narrative for this one subject with sinus bradycardia.

There are few clinically significant abnormal ECG findings. In adult patients where hypertension is common and when details of cardiac evaluation are lacking, it is difficult to determine drug causality. Cardiac findings will continue to be monitored in the postmarketing setting through safety reports and pharmacovigilance.

Effects on QT Prolongation

There was no dedicated QT study conducted, which is acceptable as pegvaliase is a large PEGylated protein which is unlikely to interact with the hERG channel to cause QT prolongation.

Immunogenicity

Refer to Section 16.4

8.2.5 Analysis of Submission-Specific Safety Issues

Anaphylaxis

A significant safety signal of high rates of anaphylaxis was identified early in the clinical development program. The Division of Pulmonary, Allergy and Rheumatology Products (DPARP) was consulted to help develop criteria to identify and adjudicate all reported cases of anaphylaxis, and provide input on the proposed risk mitigation strategies and considerations for long-term safety monitoring.

Cases were identified from the case narratives in the ISS and the CSRs from Trials 165-301 and 165-302. In addition, our DPARP colleagues performed analysis of the datasets, using MedDRA SMQs and custom queries with frequent team discussion.

The review team used a conservative approach to identify cases of anaphylaxis that met the following criteria:

- Criteria #1 from the National Institute of Allergy and Infectious Disease (NIAID) and the Food Allergy and Anaphylaxis Network (FAAN) diagnostic criteria for anaphylaxis[37]
    - Acute onset of an illness (minutes to several hours) with involvement of the skin, mucosal tissue, or both (e.g., generalized hives, pruritus or flushing, swollen lips-tongue- uvula), and at least one of the following:
        a) Respiratory compromise (e.g., dyspnea, wheeze-bronchospasm, stridor, reduced PEF, hypoxemia)
        b) Reduced BP or associated symptoms of end-organ dysfunction (e.g., hypotonia (collapse), syncope, incontinence)
- Cases which the investigator reported the adverse reaction as "anaphylaxis" or anaphylactoid reaction"
- Cases treated with epinephrine, unless there was a clear alternative etiology.

The results of the FDA internal anaphylaxis adjudication (conducted by DGIEP in consultation with DPARP) compared to sponsor's analyses are as follows:

Table 42: Summary of Adjudicated Anaphylaxis Cases (I/T/M population, N=285)

	DPARP adjudication	Sponsor's internal assessment	Sponsor's external adjudication
Number of subjects, n (%)	26 (9.1%)	33 (11.6)	13 (4.6)
Number of events	37	50	21

The team's assessment of 9.1% of subjects with anaphylaxis was less than the Applicant's initial conservative assessment of 11.6%, but more than the Applicant's external adjudicator's assessment of 4.6% in the I/T/M population. The team's assessment included 26 out of 285 subjects who had 37 anaphylactic reactions. The exposure-adjusted rate of anaphylaxis was 0.15 event rate/person-years in the induction/titration period which decreased to 0.04 event rate/person-years in the maintenance period. In the clinical trials, anaphylaxis generally occurred within 1 hour after injection (84%; 28/37 episodes); however, delayed reactions have occurred (up to 48 hours). Most episodes of anaphylaxis occurred within the first year of

[37] Sampson HA, Munoz-Furlong A, Campbell RL, Adkinson NJ, Bock SA, Branum A, et al. Second symposium on the definition and management of anaphylaxis: Summary report – Second National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network Symposium. J Allergy Clin Immunol. 2006; 117:391-7.

dosing (78%; 29/37 episodes), but cases have occurred at any time, even more than two years from initiation of treatment. Management of anaphylaxis included administration of epinephrine (54%; 20/37 episodes), corticosteroids (54%; 20/37 episodes), antihistamines (51%; 19/37 episodes), and/or oxygen (5%; 2/37 episodes). Eighteen out of the 26 (69%) patients who experienced anaphylaxis were rechallenged with pegvaliase and 5 patients had recurrence of anaphylaxis. All anaphylaxis episodes resolved without sequelae.

The mechanism of anaphylaxis appears to be mediated by immune complex/complement activation, but the specifics are unknown, as there was no predictive antibody or titer level. The mechanism is most consistent with a non-IgE Type III immune complex-mediated reaction.

The team considered all cases of anaphylaxis to be serious and severe regardless of the investigator-reported severity as anaphylaxis is generally considered a severe, potentially life-threatening, systemic allergic reaction. For cases of early recognized anaphylaxis, the symptoms may worsen if not treated emergently with injectable epinephrine or intervening medical personnel. The Applicant used Brown's criteria[38] to grade the severity of anaphylaxis. However, as the team adjudication included all anaphylaxis cases and considered those severe (as all are life-threatening), the Brown's severe criteria were not used in this review for severity categorization.

In Trial 165-301, a second protocol amendment (2014) added 3 specific risk mitigation strategies for hypersensitivity reactions, including anaphylaxis. These interventions included premedication with H1 antagonist, H2 antagonist, and/or NSAIDs approximately 2-3 hours prior to each drug dose for the duration of the titration phase of treatment. In addition, subjects were required to have a "competent adult" present with them during drug administration and for a minimum of 1 hour following administration for the first 16 weeks of the study. Finally, all subjects were required to carry epinephrine with them at all times, and received training on recognizing signs and symptoms of anaphylaxis and in self-administration of injectable epinephrine. All three risk mitigation strategies were implemented simultaneously during the phase 3 trials. As such, the potential impact of each individual intervention is difficult to determine.

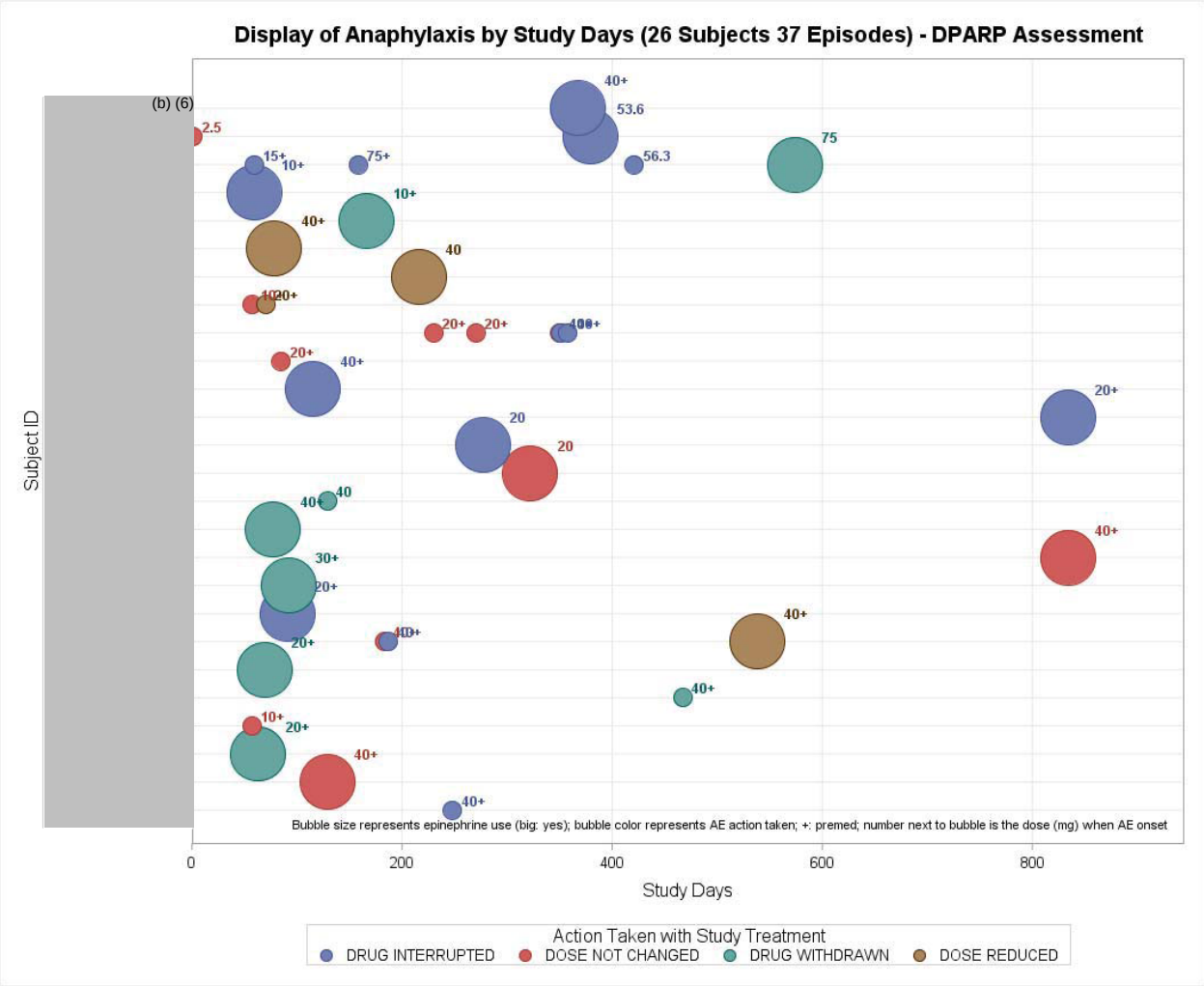
Given the anaphylaxis risk throughout pegvaliase treatment, the Applicant proposed a REMS with ETASU based on the interventions implemented in the second protocol amendment. The specifics of the REMS are discussed in more detail in the Risk Evaluation and Mitigation Strategy (REMS) section below.

Refer to the allergy consult by Dr. Stacy Chin in DARRTS, dated 3/1/2018, for further discussion.

[38] Simon GA Brown. J Allergy Clin Immunol. 2004; 114(2):371-6.

Below is a visual display of the anaphylaxis cases for comparison of timing, dose, action taken with study treatment, and epinephrine and premedication use. Bubble size represents epinephrine use (big=yes; small=no), bubble color represents AE action taken (see Figure 11 legend below), and the number next to the bubble represents the actual drug dose (mg) at AE onset.

Figure 11: Anaphylaxis Events with accompanying action taken with treatment (Trials 165-301 and 165-302)



Source: Based on BLA761079\0022\m5\datasets\iss-120-day\analysis\adam\datasets\adae.xpt and Dr. Stacy Chin's review dated 3/1/2018.

Arthralgia

Arthralgia occurred at high frequency in the clinical trials. 210 subjects (74%) had at least one arthralgia event during the induction/titration period and 137 subjects (61%) had at least one

arthralgia event during the maintenance period. The exposure-adjusted events rate was 7.64 events/person-years in induction/titration period and decreased to 1.49 events/person-years in maintenance period. Of note, more subjects had multiple events of arthralgia. Thirty-nine subjects (14%) had one event, 32 subjects (11%) had 2 events, 18 subjects (6%) had 3 events, and 146 subjects (51%) had 4 events. Arthralgia occurred as early as the first dose and at any time during treatment. The mean duration of arthralgia was 14 days and 19% of arthralgia events had a duration of at least 14 days.

As reported by the Applicant, "while the specific location on the body for AEs of arthralgia (MedDRA PT) was not systematically collected, a review of the reported verbatim terms for these events shows that the arthralgia events were widespread but more common in the extremities and back." Also reported by the Applicant was that "most of the AEs of arthralgia in the I/T/M Population were Grade 1 (756/1184, 63.9%) or Grade 2 (412/1184, 34.8%)." Severe arthralgia (severe pain, limiting self-care activities of daily living) was experienced in 14 (5%) patients.

As reported by the Applicant in the Safety population, 16 subjects experienced 23 AEs identified utilizing the search strategy for arthralgia: PTs of arthralgia, back pain, musculoskeletal pain, pain in extremity, and neck pain that were CTCAE Grade ≥ 3. Two AEs of arthralgia were reported as SAEs. The subject had a history of knee pain and meniscus injury. Treatment for the event was patellofemoral replacement and the event resolved. The other SAE was subject (b) (6) who had a medical history of right knee pain associated with a right knee dislocation and a previous left wrist fracture. The subject developed sudden onset right shoulder pain along with numbness in the right hand and fingers. This numbness was similar to what she had 1 month earlier. An MRI of the brachial plexus without contrast was reported to be grossly normal, with minimal inflammatory change at the acromioclavicular joint. An X-ray of the shoulder revealed no evidence of fracture, dislocation, periosteal reaction, or osseous destruction, and no radiographic evidence of avascular necrosis. Treatment included ketorolac and morphine and the event resolved. Causality is difficult to determine in a subject with a history of joint issues, but pegvaliase could contribute or cause the arthralgia and peripheral neuropathy as the workup did not reveal another cause. There were no Grade 4 or Grade 5 arthralgia AEs.

Arthralgia episodes were managed with concomitant medications, dosage reduction (4% of episodes), drug interruption (4% of episodes), or drug withdrawal (0.6% of episodes), and 97% of arthralgia episodes resolved by the time of the data cut-off (May 6, 2017).

In the ISS dataset, there were only a few arthritis events reported (7 events in 6 subjects), none of which were SAEs. According to the Applicant, no AEs of CTCAE Grade ≥ 3 were identified utilizing the search strategy for arthritis: MedDRA preferred terms (PTs) of arthritis, joint effusion, and joint stiffness.

Injection site reactions

Similar to other immune-mediated AEs, injection site reactions also occurred more frequency during the induction/titration period (21.89 events/person-years) compared to the maintenance period (3.95 events/person-years). As reported by the Applicant, "All but one of ISR AEs was Grade 1 or Grade 2 in severity." Also reported by the Applicant, "The most common ISR AEs (by MedDRA PT) in the I/T/M Population were injection site reaction (1859 AEs, 63.9% of all subjects), injection site erythema (722 AEs, 49.8% of all subjects), injection site bruising (301 AEs, 34.4% of all subjects), injection site pruritus (336 AEs, 32.6% of all subjects), injection site pain (264 AEs, 27.7% of all subjects), injection site swelling (255 AEs, 24.2% of all subjects), and injection site rash (194 AEs, 21.1% of all subjects)." This category includes immune-mediated and traumatic injection site reactions. The traumatic reactions are due to SQ administration technique rather than drug induced.

The exposure-adjusted event rate was about twice as high in the 20 mg/day group (6.81 events/person-years) compared to the 40 mg/day (3.06 events/person-years) of those subjects who reached the maintenance period. Of note, the placebo group had an exposure-adjusted event rate close to the 40 mg/group of 2.35 events/person-years. This is likely due to traumatic reactions contributing significantly to injection site reaction AEs.

Three injection site reactions consistent with granulomatous skin lesions were reported. One subject ((b) (6)) exclusively injected in the thighs and was diagnosed with xanthogranuloma on the bilateral thighs at previous injection sites. The subject withdrew from study drug as a result of this AE and received treatment with adalimumab. The event resolved after 638 days.

Subject ((b) (6)) was diagnosed with necrobiosis lipoidica diabeticorum at the injection site which lasted 281 days. The subject continued to receive daily injections and was seen by a dermatologist who diagnosed ulceration with focal breakdown. This wound was complicated by *Pseudomonas aeruginosa* infection.

Subject (b) (6) developed granulomatous dermatitis that resulted in an unspecified procedure which resolved 16 days later. This subject continued on pegvaliase.

Serum Sickness

In the I/T/M population, there were 7 events with reported PTs of serum sickness in 7 subjects. Serum sickness episodes were more frequent during the induction/titration period (0.04 event/person-years) and decreased during the maintenance period (less than 0.01 events/person-years). Five of the 7 patients continued treatment without a recurrence and managed serum sickness with drug interruption, dosage reduction, and/or concomitant medication. 2 subjects discontinued treatment. Both events were reported as SAEs. Treatment included diphenhydramine and steroids and the events resolved.

PEG exposure

In the context of the relatively high PEG content of PALYNZIQ as shown in Table 43 (~9 molecules of 20KDa PEG per pegvaliase monomer), the review team discussed internally and with the Applicant as well as evaluated in detail for any evidence of clinical effects of PEG exposure during the phase 3 trials. No evidence for major organ dysfunction (such as renal disease) was found during PALYNZIQ treatment in the phase 3 trials after a mean exposure of 2 years (and an exposure of over 4 years in some patients). The potential clinical effects of chronic PEG exposure in the context of chronic use of other approved PEGylated products are unclear and those effects, if any, with chronic PALYNZIQ treatment are also unknown. As such, any potential long-term clinical effects of PEG exposure and/or PEG deposition in major organs (e.g., kidneys) will be evaluated prospectively in the post-marketing setting over a treatment exposure of at least 10 years. Refer to section 5, nonclinical pharmacology/toxicology, for additional details regarding assessment of PEG-related effects in nonclinical studies.

Table 43: PEG content (mg) by Pegvaliase Dose

Pegvaliase dose (mg rAvPAL)	Average mg amount of PEG
2.5	7.3
10	29
20	58
40	117

8.2.7 Safety Analyses by Demographic Subgroups

Table 44: Adverse Events by Sex (I/T/M population, N=285)

	Male (n=142)		Female (n=143)	
	Incidence (%)	Events/ Event Rate	Incidence (%)	Events/ Event Rate
Total treatment exposure (person-years)		**248.0**		**225.4**
AEs				
Any AE	142 (100%)	5050 (20.37)	143 (100%)	8902 (39.49)
AEs assessed by investigator as related to pegvaliase	142 (100%)	3265 (13.17)	143 (100%)	5988 (26.57)
AEs leading to dose reduction	37 (26.1%)	74 (0.30)	54 (37.8%)	169 (0.75)
AEs leading to dose interruption	46 (32.4%)	163 (0.66)	51 (35.7%)	137 (0.61)
AEs leading to pegvaliase discontinuation	14 (9.9%)		27 (18.9%)	
AEs leading to study discontinuation	9 (6.3%)		17 (11.9%)	
Neuropsychiatric AEs	46 (32.4%)	117 (0.47)	68 (47.6%)	228 (1.01)
SAEs	24 (16.9%)	32 (0.13)	28 (19.6%)	37 (0.16)
AEs with CTCAE Grade ≥ 3	28 (19.7%)	42 (0.17)	34 (23.8%)	50 (0.22)
Deaths	1 (0.7%)		0	
AEs of special interest/significance				
HAEs	128 (90.1%)	1237 (4.99)	138 (96.5%)	2281 (10.12)
Anaphylaxis (NIAID/FAAN)	16 (11.3%)	29 (0.12)	17 (11.9%)	21 (0.09)
Anaphylaxis (external expert)	6 (4.2%)	12 (0.05)	7 (4.9%)	9 (0.04)
Injection-site reactions	123 (86.6%)	1494 (6.03)	140 (97.9%)	2996 (13.29)
Injection-site skin reactions ≥ 14 days	54 (38.0%)	115 (0.46)	65 (45.5%)	224 (0.99)
Generalized skin reactions ≥ 14 days	52 (36.6%)	92 (0.37)	58 (40.6%)	109 (0.48)
Arthralgia	85 (59.9%)	385 (1.55)	118 (82.5%)	799 (3.54)

Source: Applicant's submission, dated 6/30/2017, Module 5.3.5.3 Integrated Summary of Safety, page 236.

As illustrated in the Table 44, AEs were reported with greater frequency by females as compared to males in the phase 3 trials. Overall, the exposure-adjusted rates of AEs appeared to be balanced between males and females with the exception of neuropsychiatric AEs, HAEs, injection-site reactions, injection site reactions lasting over 14 days, and arthralgias which were reported with a higher frequency in females than in males. This imbalance cannot be explained

based on any differences in disease pathology or other biological factors. Further characterization of potential differences between females and males with PKU in terms of AE incidence during pegvaliase treatment will be further assessed through the post-approval studies.

8.2.9 Additional Safety Explorations

Human Carcinogenicity or Tumor Development

Not applicable.

Human Reproduction and Pregnancy

Based on the 120-day safety update report and cumulative pregnancy data, 10 female subjects and 9 female partners of male study subjects became pregnant during treatment. Two male subjects have female partners who were pregnant twice, for a total of 11 partner pregnancies.

As cited by the Applicant, 3 pregnancies were associated with SAEs.

Table 44: Pregnancy-Associated Serious Adverse Events

Table 10.4.2.1: Pregnancy-Associated Serious Adverse Events (MD Population)

SAE Preferred Term	Gestational Weeks at Time of Event	Dose and Frequency at Time of Event	Number of Doses Received post-LMP
Stillbirth	36 weeks and 6 days	40 mg QD	34
Abortion missed	11 weeks and 3 days	2.5 mg QW	3
Abortion induced	Unknown (34 days after LMP)	1 mg/kg 3x/week	11

LMP, last menstrual period; MD, Multiple Dose (Population); QD, once daily; QW, once weekly.

Source: Listing 2.7.4.3.2

Source: Applicant's submission, dated 10/31/2017, Module 5.3.5.3 120 Day Safety Update Table 10.4.2.1: Pregnancy-Associated Serious Adverse Events (MD Population), page 262.

These SAEs are described briefly below, with reference to the 120 Day Safety Update, page 262:

- A 26-year-old female who discontinued treatment with pegvaliase five weeks after her last menstrual period (LMP). The subject's most recent Phe level was within her goal range. At gestational age 36 6/7 weeks, she began leaking vaginal fluid and no fetal movement was detected. The subject delivered a non-viable male fetus. Placenta

examination showed velamentous insertion of the cord, and some clot adherent to the maternal side of the placenta. The investigator reported the SAE of stillbirth as not related to treatment with pegvaliase and was due to probable placental abruption.

- A 20-year-old female who received 3 doses of pegvaliase after her LMP and then discontinued treatment. An ultrasound performed at around 11 weeks after her LMP revealed no fetal heartbeat and a missed abortion. The subject had elevated blood Phe levels and the investigator reported that the missed abortion could be possibly related to pegvaliase.
- An 18-year-old female with exposure to pegvaliase prior to and during the first month following conception. The subject had a therapeutic abortion.

From the 120 Day Safety Update, page 263:
Of the 7 remaining pregnancies:

- "One resulted in the full-term birth (40 gestational weeks) of an infant with a Grade 1 systolic murmur (resolved Day 2 of life) and neonatal pustular melanosis (assessed by the investigator as non-serious); the subject discontinued pegvaliase 2 doses after LMP.
- One resulted in the full-term birth (39 gestational weeks) of a healthy baby: the subject received 10 mg/day pegvaliase during her pregnancy (46 doses received post LMP).
- Two subjects underwent therapeutic abortions.
- Three pregnancies were ongoing at the end of the Safety Update Period.

In the 11 partner pregnancies (defined as female partners of male subjects who became pregnant), 6 pregnancies had a reported normal outcome. For the other 5 pregnancies:

- One subject's partner delivered a neonate, but no additional details were available.
- One subject's partner declined to provide information about her pregnancy.
- One subject's partner became pregnant during the subject's Screening period for Trial 165-301; the subject had received no doses of pegvaliase prior to the pregnancy. The subject was later lost to follow-up, and the outcome of the pregnancy is not known.
- One partner pregnancy was ongoing at the end of the Safety Update Period.
- One partner pregnancy was associated with neonatal respiratory distress. Subject (b) (6) received pegvaliase treatment prior to conception, and continued to receive pegvaliase during the pregnancy. The subject's partner gave birth to the infant (4.09 kg) at 40 weeks of gestation (APGAR 4/7 at 1 and 5 minutes). Delivery complications included low-grade maternal fever (99.1-99.6°F), nuchal cord, meconium-stained amniotic fluid, and fetal tachycardia during the last 30 minutes of second stage labor. The neonatal course was complicated by respiratory distress following weaning off of respiratory assistance. The infant was admitted to the NICU. The infant required respiratory and nutritional support and then was discharged to home 2 days after birth."

There is limited data on the developmental effects of pegvaliase use in pregnant woman. In summary, the 10 pregnancies included 3 therapeutic/induced abortions, 1 missed abortion, 1 stillbirth, 1 normal delivery, 1 delivery of an infant with transient systolic murmur which resolved without intervention, and 3 ongoing at the time of the Safety Update data cutoff. It is

known that pregnant patients with PKU are at increased developmental risk with elevated Phe levels, so causality can be difficult to establish with limited subject details and lab data. There will be a pregnancy pharmacovigilance program for pegvaliase. It is discussed in the label that pegvaliase may cause fetal harm with supporting animal and human data, although the data is limited and insufficient to determine a drug-associated risk of adverse developmental outcomes.

Pediatrics and Assessment of Effects on Growth

Not applicable.

Overdose, Drug Abuse Potential, Withdrawal, and Rebound

Not applicable.

8.2.11 Integrated Assessment of Safety

The most common AEs experienced by the 285 patients of the I/T/M population included injection site reactions, arthralgia, hypersensitivity reactions, headache, and generalized skin reactions lasting at least 14 days. The exposure-adjusted event rates of all AEs were highest during the induction/titration period (Trial 165-301) and decreased in the maintenance period (Trials 165-301 and 165-302). The exposure-adjusted AE rates in the 20 mg once daily and 40 mg once daily treatment groups were similar. There was higher exposure-adjusted event rates for some hypersensitivity events in the treated females as compared to the treated males but no specific explanation for this imbalance is readily apparent (which may be partly due to better reporting of AEs by females); this will be further assessed in the post-approval safety studies.

Anaphylaxis occurred in 9% of subjects in the I/T/M population based on FDA internal adjudication. To mitigate the potential life-threatening complications associated with anaphylaxis, measures that will be taken in the postmarketing setting include a Boxed Warning in the product label and a REMS with ETASU, which will include prescriber certification, pharmacy certification, patient education, patient enrollment, and prescription for auto-injectable epinephrine to be carried by treated patients at all times during pegvaliase treatment.

There was an association between anti-drug antibodies and occurrence of AEs. The highest frequency of hypersensitivity AEs occurred within the first 6 months of pegvaliase treatment when the mean circulating immune complex concentrations were at their peak and mean C3 and C4 levels were at their nadir. Based on this parallel timing, the most likely mechanism of the hypersensitivity reactions is a Type III, immune complex-mediated hypersensitivity. Based on review of clinical and laboratory safety data, the team has not identified any clinical or laboratory evidence of immune complex or PEG-mediated kidney or other organ damage during pegvaliase treatment over a median exposure of 2 years. In a small proportion of patients who had follow up measurements, sustained high anti-drug antibody titers persisted and mean concentrations of circulating immune complexes and complement C3 and C4 factors were not back to baseline levels over long-term treatment, necessitating continuous assessment of immunogenicity and its impact on safety in the postmarketing setting.

In the rabbit embryo-fetal development study, there was a high incidence of embryo lethality, fetal growth restriction, and fetal malformations (skeleton, kidneys, eyes, lungs) in the setting of concomitant significant maternal toxicity. Although a clear explanation for these findings is not apparent, it is conceivable that the fetal malformations and poor fetal outcomes may be due to maternal Phe depletion which may have caused the maternal toxicity (which will be further evaluated in a post-approval nonclinical study of induced maternal Phe depletion without pegvaliase treatment). Also, the Applicant will conduct a postmarketing prospective

observational study to evaluate pregnancy complications and fetal/newborn outcomes in pregnant women with PKU treated with pegvaliase.

Overall, pegvaliase treatment demonstrated an acceptable benefit vs risk profile with precautions in place to mitigate the serious safety risks through labeling and the REMS with ETASU program in conjunction with postmarketing requirements to further investigate and characterize long-term safety risks associated with pegvaliase treatment in adult patients with PKU.

8.3 Summary and Conclusions

8.3.1 Statistical Issues

There were no major statistical issues affecting the overall conclusion. Based on Trial 165-302 Part 2, both 20 mg/day and 40 mg/day target doses of pegvaliase were statistically significantly better than placebo in maintaining blood Phe concentration in terms of mean change in blood Phe concentration from Part 2 Baseline to Part 2 Week 8. The magnitude of benefit in these two treatment arms was similar. The efficacy of pegvaliase during Trial 165-302 Part 2 was consistent among subgroups.

In Trials 165-301 and 165-302 Part 2, there were extremely low blood Phe concentrations (≤ 30 micromol/L) noted in both treatment arms, more frequently in the patients dosed continuously at 40 mg/day compared to those dosed at 20 mg/day. The mean blood Phe concentration appeared to be driven by these extremely low values.

Patients in both Trials 165-301 and 165-302 were instructed to maintain a consistent diet. Only 2 patients in Trial 165-301 and 1 patient in Trial 165-302 maintained the protocol-defined-consistent diet. Thus, the observed Phe reductions in these trials occurred in association with an unrestricted diet.

We note the following limitations:

1. 86 out of 273 (31.5%) randomized subjects were included in the primary analysis in Trial 165-302 Part 2. It is not clear whether they adequately represent the target population.
2. Patients' responses to the study drug were variable. Some patients responded to the low dose while others did not response to the study drug at all. Efforts to identify responders based on patients' baseline characteristics were limited due to a lack of systematic collection of genetic information. Thus, potential predictors of response could not be identified from patients' genetics.

3. Some patients showed a lowering of blood Phe concentration at very low doses of pegvaliase. However, due to the limitation of the trial design, the efficacy of lower doses could not be fully assessed.
4. In Trial 165-301, the benefit of pegvaliase 20 mg/day was not fully evaluated before the subject was titrated up to 40 mg/day. The median time for patients reaching 40 mg/day from 20 mg/day was only 7 days.

8.3.2 Conclusions and Recommendations

Pegvaliase at target maintenance doses of 20 mg once daily and 40 mg once daily reduced blood Phe concentrations in treatment-naïve patients with PKU in Trial 165-301. The recommended titration scheme from 20 mg/day to 40 mg/day in the product label was not formally tested as a pre-specified statistical analysis in the phase 3 trials, but analyses of data from patients who were treated continuously with 20 mg once daily in Trials 165-301 and 165-302, part 4 (open label extension) show that some patients who did not reach a therapeutic response on the 20 mg/day dose did respond when escalated to the 40 mg/day dose. The benefit of the target dose of 20 mg/day should be carefully evaluated before patients are titrated to 40 mg/day given the identified safety risks associated with treatment. In Trial 165-302 Part 2 (placebo-controlled, randomized withdrawal), both the 20 mg/day and 40 mg/day arms maintained their blood Phe concentrations compared to patients who were switched to placebo. In Trials 165-301 and 302 Part 2, hypophenylalaninemia (blood Phe concentration ≤ 30 micromol/L) was observed in both treatment arms with a slightly higher incidence in the patients dosed continuously at 40 mg/day arms. It appeared that these low blood Phe values may have driven the mean blood Phe concentration down.

Overall, the totality of data in the pegvaliase clinical development program demonstrated clinically and statistically significant Phe reduction in uncontrolled adult PKU patients on a largely unrestricted diet. The administration of pegvaliase through an induction, titration, and maintenance dosing regimen was generally efficacious. The data show an acceptable safety profile with precautions in place to mitigate the serious hypersensitivity and anaphylaxis safety signals through labeling and the REMS program. As such, we recommend approval of pegvaliase with a REMS to reduce blood Phe levels in adult patients with PKU who have uncontrolled blood Phe levels > 600 micromol/L on existing management.

9 Advisory Committee Meeting and Other External Consultations

The application for PALYNZIQ was not referred to an FDA advisory committee because outside expertise was not necessary; there were no controversial issues that would benefit from advisory committee discussion.

10 Pediatrics

This section is not applicable to this BLA as the studied population was adults and the drug obtained orphan drug designation on March 8, 1995 (designation #95-0881). Thus, this BLA is exempt from PREA requirements.

11 Labeling Recommendations

11.1 Prescription Drug Labeling

The pegvaliase label includes instructions on gradual dose titration from a starting dose of 2.5 mg once weekly up to the lowest effective maintenance dose based on therapeutic response (defined as a ≥20% blood Phe reduction from baseline or blood Phe ≤600 micromol/L) and patient tolerability. Dose increase from 20 mg once daily to 40 mg once daily is recommended in patients who do not achieve a therapeutic response while on continuous treatment with 20 mg once daily for at least 24 weeks. In addition, pegvaliase discontinuation is recommended in patients who do not achieve a therapeutic response after an additional 16 weeks of continuous treatment with 40 mg once daily. This detailed dosing and discontinuation recommendations will enable informed decisions about dose titration while at the same time minimizing the exposure to the drug (with its associated immune-mediated adverse reactions) in patients who are not deriving benefit after a sufficient duration of treatment. Blood Phe concentrations should be monitored and dietary intake should be modified as well as the pegvaliase dose reduced in the event of hypophenylalaninemia (blood Phe concentration below 30 micromol/L). A Box Warning is included regarding the serious risk of anaphylaxis. Premedication with H1-receptor or H2-receptor antagonists and/or antipyretics may be considered for hypersensitivity reactions. An adult observer may be considered for patients who are unable to adequately recognize and manage anaphylaxis. Please refer to the final agreed-upon label for details of the prescribing information.

11.2 Patient Labeling

An Instructions for Use document is included which describes in detail pegvaliase self-administration for patients. Please see final, agreed-upon Instructions for Use document for full details. Also, a Medication Guide highlights the most important administration and safety information for patients.

12 Risk Evaluation and Mitigation Strategies (REMS)

Pegvaliase is associated with high rates of hypersensitivity AEs (93%) and anaphylaxis (9%). The proposed labeling for pegvaliase has been maximized and includes a Boxed Warning, Medication Guide, a narrow indication to those who have uncontrolled blood Phe levels on existing management, a maximum recommended dose, and discontinuation for lack of efficacy. Further risk mitigation in the form of a REMS is necessary to ensure that prescribers and patients are aware of the risk of anaphylaxis and to ensure safe use requirements are met before administration of pegvaliase. Auto-injectable epinephrine must be prescribed to all patients receiving pegvaliase and should be readily available at all times during pegvaliase treatment. The proposed labeling recommends co-prescribing of auto-injectable epinephrine in the Warning and Precautions section and Boxed Warning, however, the additional requirement provided by a REMS will ensure that all patients have access to auto-injectable epinephrine while on pegvaliase therapy.

Due to the concerning anaphylaxis safety signal, the Applicant proposed a REMS with ETASU based on the interventions implemented in the second protocol amendment (Section 8.2.5 Analysis of Submission-Specific Safety Issues, Anaphylaxis). BioMarin submitted a complete REMS proposal including a REMS document, supporting document and appended materials with their original BLA submission dated June 30, 2017. The REMS proposal contained a (b) (4) prescriber certification (ETASU A), pharmacy certification (ETASU B), the requirement that pegvaliase should not be dispensed to patients without documentation of safe use conditions (ETASU D), an implementation system, and a timetable for submission of assessments. The safe use conditions were defined as patient enrollment and counseling, auto-injectable epinephrine, premedications (b) (4)
(b) (4)

The safety concern of anaphylaxis and the Applicant's proposed REMS were discussed on December 13, 2017 at the meeting of the REMS Oversight Committee (ROC)[39]. The ROC concurred that additional risk mitigation measures beyond labeling including a REMS with ETASU are necessary to ensure the benefits of pegvaliase outweigh the serious risk of anaphylaxis. Labeling alone will not ensure that prescribers and patients are informed of the risk of anaphylaxis and that patients have access to auto-injectable epinephrine prior to starting pegvaliase. A REMS will ensure that both the prescriber and patient are informed about the risk of anaphylaxis. The REMS will be used to educate patients on how to recognize and respond to signs and symptoms of anaphylaxis and ensure that they have auto-injectable epinephrine as a

[39] As per the 21st Century review process, all REMS with elements to assure safe use (ETASU) are discussed at the REMS Oversight Committee (ROC), which consists of senior-level management from the Offices of New Drugs, Surveillance and Epidemiology, and Regulatory Policy.

safe use condition. Auto-injectable epinephrine must be prescribed to all patients receiving pegvaliase and should be readily available at all times during pegvaliase treatment.

The minimum necessary elements required include:

1. Prescriber certification (ETASU A) to ensure that each prescriber is informed of the risk of anaphylaxis, the need to counsel patients about the risk, and the need to prescribe auto-injectable epinephrine.
2. Pharmacy certification (ETASU B) to ensure that patients and prescribers are enrolled or certified in the program and thus aware of the risk of anaphylaxis, and to verify that the patient has auto-injectable epinephrine prior to dispensing the product.
3. Safe-use conditions (ETASU D) include patient enrollment to ensure that each patient is counseled and trained on how to recognize and respond to anaphylaxis and that each patient has auto-injectable epinephrine available at all times.

(b) (4) Including the MG as a requirement is not necessary as patient materials have been developed specific to the REMS risk. The MG will be (b) (4) a part of the approved labeling and will be dispensed with each PALYNZIQ prescription in accordance with 21CFR 208.24.

The appended materials will include: a prescriber enrollment form, prescriber guide, prescriber knowledge assessment, a program overview, pharmacy enrollment form, patient enrollment form, patient guide, safety video, wallet card, and a website. Enrollment forms will include attestations for each relevant stakeholder regarding knowledge of the REMS risk, as well as the safe use conditions required before dispensing and administration of the product. The prescriber knowledge assessment will ensure that prescribers understand the key risk messages included in the prescriber training at the time they take the assessment.

The REMS Program Overview will be a concise document for any stakeholder to reference for REMS program operations and requirements. Patient directed materials include a wallet card, a safety video, and a patient guide to present information about the risks and what to do in the event they experience anaphylaxis, and that they must have auto-injectable epinephrine available at all times during treatment. Finally, a REMS website will assist in operationalizing the program by having all materials available and having online enrollment available. Further, an implementation system and timetable for submission of assessments will be requirements included in this REMS.

Due to uncertainty of its effect on the anaphylaxis rate in clinical trials and the questionable real-world feasibility, the (b) (4) (b) (4) Agency concluded that the use of monitoring by a trained observer (b) (4) will not mitigate the risk of anaphylaxis for patients using pegvaliase (b) (4) (b) (4)

(b) (4) Other allergic conditions such as severe food allergies, bee sting allergies, or other drug allergies do not require an observer and are also unpredictable much like the anaphylactic events seen in the pegvaliase clinical trials. Although the use of an observer was recommended by the review team to incorporate into the clinical trials in response to anaphylactic events, after further review of the data, at this time, the Agency does not recommend that use of an observer should be required for all patients taking pegvaliase and does not recommend it be included as a safe use condition in the REMS. The Agency's current thinking is that the need for an observer should be determined by the healthcare provider based on individual patients' needs.

Similarly, due to uncertainty of their effect on the anaphylaxis rate in clinical trials and the questionable real-world feasibility, (b) (4) premedications will not be included as a requirement in the REMS. Although the use of premedications may provide symptomatic relief of hypersensitivity-associated clinical findings such as rash, itching, and fever, the benefits do not outweigh the risks of required premedications for all patients using pegvaliase and anaphylaxis events continued to occur despite patients using premedications. The Agency is concerned that the long-term use of the recommended pre-medications, specifically non-steroidal anti-inflammatories (NSAIDS), is associated with serious safety risks including gastrointestinal ulcer, cardiovascular risk, and bleeding. Additionally, the use of multiple medications may mask the early signs of hypersensitivity or anaphylaxis as well as may result in pill burden for patients. After further review of the data, at this time, the Agency does not recommend that premedications should be required for all patients taking pegvaliase and does not recommend that they be included as a safe use conditions in the REMS. The Agency concludes that it should be per the prescriber's digression whether their patient could benefit from the use of premedications and specifically which premedications to use.

We have reviewed the following REMS Program materials submitted by BioMarin:

- REMS Document
- Prescriber Enrollment Form
- Patient Enrollment Form
- Pharmacy Enrollment Form
- Prescriber Guide
- Prescriber Knowledge Assessment
- Patient Guide: What You Need To Know
- Safety Video Transcript and Storyboard
- Wallet Card
- Program Overview
- Website Screenshots
- Supporting Document

Reviewer Comments: *When initially submitted, the Applicant did not include the following REMS materials: a Pharmacy Enrollment Form to ensure that pharmacies who wish to enroll in the REMS program document their agreement to the requirements via attestations, a Program Overview to explain the role of each stakeholder in the REMS, a patient directed Safety Video to further convey the risk of anaphylaxis to patients and their role in the REMS, and a Prescriber Knowledge Assessment to ensure that prescribers understand the risk of anaphylaxis and their role in the REMS. The Agency informed the Applicant of these requirements, and they were included in the May 22, 2018 submission. All other initially submitted materials were agreed to be necessary by the Agency. Further, the Applicant has included key risk messages that need to be conveyed.*

The Applicant proposes to submit assessments to the FDA at 6 months and 12 months post approval of the REMS and annually thereafter from the date of the initial approval of the Palynziq REMS. The assessment plan must include, but is not limited to, the following:

1. PALYNZIQ REMS Implementation (6-month and 12-month assessment only)
    a. Product launch date
    b. Date when the PALYNZIQ REMS website became active and is fully operational
    c. Date prescribers could become certified online, by mail, or by fax
    d. Date when the REMS call center is fully operational
    e. Number of unique visits to the PALYNZIQ REMS website during the assessment period
2. Post-Training Prescriber Knowledge Assessments (KA) (6-month and 12-month assessment only)
    a. Number of completed post-training knowledge assessments for healthcare providers including methods of completion and number of attempts to complete
    b. Summary of the most frequently missed KA questions
3. PALYNZIQ REMS Enrollment Statistics (per reporting period and cumulatively)
    a. Healthcare Providers
        i. Number of newly enrolled and active (have prescribed PALYNZIQ at least once during the reporting period) prescribers with profession (physician, advance practice nurse, physician assistant, etc.) and specialty
    b. Pharmacies/Distributors
        i. Number of newly enrolled and active (existing/dispensed a shipment of PALYNZIQ) distributors/certified pharmacies with pharmacy type
    c. Patients

i. Number of newly enrolled and active (have received at least one shipment of PALYNZIQ during the reporting period) patients with demographics (age and gender)

d. The number of patients/healthcare providers/pharmacies/distributors that were de-enrolled and the reason for de-enrollment

4. PALYNZIQ Utilization Data (per reporting period and cumulatively)
    a. Number of PALYNZIQ prescriptions (new and refills) dispensed stratified by:
        i. Pharmacy Type
        ii. Healthcare Provider specialty
        iii. Patient demographics (age and gender)
5. REMS Infrastructure and Performance (current reporting period and cumulatively)
    a. PALYNZIQ REMS Call Center Report
        i. Number of contacts by stakeholder type (patient/, healthcare provider, pharmacy, distributor, other)
        ii. Summary of frequently asked questions (FAQ) by stakeholder type
        iii. A summary report of corrective actions resulting from issues identified
6. Safety Surveillance
    a. Adverse event assessments of anaphylaxis
        i. Include the search strategy used to identify cases (via safety database) and specific MedDRA terms used to identify cases of interest
        ii. Include a line listing of all cases that includes: manufacturer control number, narrative, and assessment of causality
    b. A study to evaluate prescriber's adherence to the need to prescribe auto-injectable epinephrine with PALYNZIQ.
7. REMS performance/compliance

1. Audits: Summary of audit activities conducted during the reporting period including but not limited to
    a. An overview of the audit plan for each stakeholder
    b. The number of audits performed
    c. A summary report of the processes and procedures that are implemented in order to be in compliance with the PALYNZIQ REMS requirements
    d. A summary report of deviations found, associated corrective and preventive actions (CAPA) plans, and the status of CAPA plans
2. Number of prescribers and pharmacies and distributors de-certified and reasons for decertification and actions to address non-compliance
3. Number of PALYNZIQ prescriptions dispensed that were written by non-certified prescribers and any action taken and outcome of action (e.g., provision of educational materials, prescriber became certified)
4. Number of PALYNZIQ prescriptions dispensed by noncertified pharmacies and the actions taken to prevent future occurrences

5. Number of PALYNZIQ prescriptions dispensed to de-enrolled or non-enrolled patients , sources of report, and actions taken to prevent future occurrences
6. Number of patients who received PALYNZIQ without access to auto-injectable epinephrine
7. Number of times a PALYNZIQ prescription was dispensed because a certified pharmacy bypassed REMS authorization processes, to include a description of how the events were identified and any corrective actions taken.
8. Number of shipments sent to non-certified pharmacies, sources of the reports, and actions taken to prevent future occurrences
9. Summary of any additional non-compliance, source of report, resulting corrective and preventive actions (CAPA)

8. Evaluation of Knowledge (beginning with the 12-month assessment)
    a. Patient understanding of:
        i. How to recognize and respond to signs and symptoms of anaphylaxis
        ii. The need to carry auto-injectable epinephrine with them at all times
    b. Healthcare provider understanding of:
        i. The risk of anaphylaxis
        ii. The need to counsel patients about the risk of anaphylaxis and how to recognize and respond to signs and symptoms of anaphylaxis
        iii. The need to enroll patients in the PALYNZIQ REMS
        iv. The need to prescribe auto-injectable epinephrine with PALYNZIQ
9. The requirements for assessments of an approved REMS under section 505-1(g)(3) include with respect to each goal included in the strategy, an assessment of the extent to which the approved strategy, including each element of the strategy, is meeting the goal or whether one or more such goals or such elements should be modified.

The risk of anaphylaxis associated with pegvaliase is serious. Prescribers must understand this risk, the importance of patient counseling and ensure that the patient has access to auto-injectable epinephrine. Based on the magnitude and severity of the risk of anaphylaxis, a REMS with ETASU is necessary to ensure that the benefits outweigh the risks. The proposed REMS as submitted on May 22, 2018 is acceptable is appended to the approval letter.

For more information, refer to the final DRISK review finalized on May 24, 2018 (Reference ID: 4268095) and the REMS Memorandum to File finalized by Dr. Joyce Korvick on May 24, 2018 (Reference ID: 4268233).

13 Postmarketing Requirements and Commitments

Given the high frequency of immune-mediated adverse reactions in the pegvaliase phase 3 trials as well as the finding of fetal malformations identified in the nonclinical studies, post-marketing requirements (PMRs) were agreed upon with the sponsor in order to further evaluate and characterize:

1. the incidence of severe immune-mediated adverse reactions (including anaphylaxis) and of immunologic and inflammatory responses (e.g., antibodies, circulating immune complexes, complement C3 and C4 levels, CRP), and their potential chronic effects on major organ function (e.g., kidneys);
2. the impact of the immune responses on therapeutic response (blood Phe concentrations) and on the rates of severe adverse reactions;
3. the immune responses to pegvaliase by improving the existing assays for IgM, IgG, and IgE antibody detection to the PAL protein and the PEG molecule and testing blood samples from patients enrolled in the long-term observational study (#1 above);
4. the ability of an immune tolerance induction (ITI) regimen given prior to or concurrently with pegvaliase to suppress immune response, to reduce the risk of severe immune-mediated adverse events, and to enable improved therapeutic response;
5. pregnancy and fetal/newborn outcomes in women with PKU treated with pegvaliase; and
6. the mechanism of fetal malformations via an animal study of maternal Phe depletion in rabbits during pregnancy (and without pegvaliase treatment).

14 Division Director (DGIEP)

I agree with the recommendation for approval of PALINZQ for the indication of "reducing blood phenylalanine concentrations in adult patients with phenylketonuria who have uncontrolled blood phenylalanine concentrations greater than 600 micromol/L on existing management."

PKU is a disease with limited therapeutic options. While diet is a fundamental treatment tool across all age groups (and certainly essential in the treatment of children, in whom it prevents the devastating occurrence of mental retardation), it has a more limited impact in adults as many fail to comply with daily dietary instructions. In addition, adults with PKU oftentimes enter a vicious cycle that starts with lack of compliance to diet, followed by exacerbation of hyperphenylalaninemia, the development or worsening of psychiatric/neurologic symptoms (impaired executive functions, attention and memory; and even anxiety, depression) that further impact patients' effective participation in the management of their own complex diet. The only other therapeutic option in PKU is Kuvan, whose effectiveness is limited to a subgroup of adult PKU patients.

This application has provided evidence that pegvaliase is effective in lowering blood Phe in adults with PKU. In Trial 165-301, in a patient population of adult PKU patients who were not metabolically controlled on diet (patients entered the trial with a baseline Phe of 1,221 micromol/L), both pegvaliase doses evaluated (20 mg and 40 mg) lowered blood Phe. While not bringing all subjects to the desired target (<600 micromol/L), pegvaliase did it successfully in about 1/3 of all patients. In some patients, pegvaliase treatment reduced blood Phe levels to the limit of detection of the assay. While overtreatment is not desirable, this issue can be addressed in a clinical practice setting by active management of the diet and relaxing the Phe intake, which could not be done during the trial because patients were required to maintain a relatively constant protein intake. Of note, in this trial, 84% of patients were on an unrestricted diet prior to pegvaliase treatment initiation.

In a second trial (165-302), in two independent substudies conducted in pegvaliase responders, patients on 20 mg once daily or 40 mg once daily were randomly assigned to either placebo or continuation of the respective dose. Patients who were switched to placebo returned to their pre-treatment Phe concentrations after only 8 weeks of treatment withdrawal, while pegvaliase treated patients maintained the same level of Phe control.

I agree with approving and labeling both the 20 mg and 40 mg dose as both demonstrated similar efficacy. From the analysis of the safety data, there was no clear advantage for the 20 mg dose over the higher 40 mg dose. In addition, the 40 mg dose seemed to confer a slight therapeutic advantage as some patients who did not respond well on 20 mg, when the dose was increased, had further reductions in blood Phe.

Two distinct safety risks were associated with the use of pegvaliase: 1) hypersensitivity reactions (including anaphylaxis), and 2) a robust immunogenic response (including sustained elevations in anti-drug antibodies, low serum complement levels, and circulating immune complexes). Although observed with relatively high frequency at the initiation of pegvaliase treatment, allergic reactions are not unusual for biologics; in addition, the frequency of allergic reactions decreased to some extent with subsequent use. Of interest, patient interviews indicated that this risk is acceptable to many of them in the light of the control of Phe. This observation seems to be supported by the high rate of compliance in the trial, despite such adverse events and the discomfort associated with daily subcutaneous injections.

Immunogenicity remains a matter of concern as most patients developed a sustained immune response. The clinical consequences that were observed for the duration of the clinical program (2 years on average, up to 4 years or longer in some patients), were manageable, and there was no evidence of severe immune complex related disease such as changes in renal function, development of progressive or persistent proteinuria, or immune complex-mediated glomerulonephritis. However, the long-term safety of pegvaliase needs to be further characterized post-approval. Therefore, I agree with the implementation of a REMS with ETASU so that patients who could benefit from pegvaliase treatment are appropriately selected by the prescribing physicians, and patients are informed and educated about the risk of allergic reactions and trained on how to self-administer epinephrine with an autoinjector. I am also in full agreement with the postmarketing studies, which will evaluate long-term safety as well as the feasibility of an immune tolerance regimen which may help mitigate the immunogenic risk.

Pegvaliase can make an important contribution to the management of adults with PKU. When carefully titrated in conjunction with dietary management, it can help bring blood Phe levels in the currently recommended target range in a large proportion of PKU patients. Used appropriately, it can also help relax dietary management and allow patients access to a more diverse diet, which is in itself a desirable outcome. Although the pegvaliase clinical program did not evaluate formally or extensively the benefit on neuropsychiatric endpoints, one also needs to acknowledge that in PKU the standard of care and goal of treatment is metabolic control measured by reduction on Phe levels, and this goal is clearly articulated in current clinical practice standards and guidelines.

15 Office Director (ODE III)

I concur with the recommendation of the Division of Gastroenterology and Inborn Errors Products to approve PALYNZIQ (pegvaliase-pqpz) with a REMS to reduce blood Phe concentrations in adult patients with PKU who have uncontrolled blood Phe concentrations > 600 micromol/L on existing management. PALYNZIQ will be administered using an induction/titration/maintenance regimen to a recommended target dose of 20 mg/day. PALYNZIQ is an original biologic product.

The overall goal of treatment for PKU patients is reduction in blood Phe concentration. Control of blood Phe concentrations within the recommended therapeutic range is particularly challenging for adults with PKU due to the difficulty of adhering to dietary restrictions of daily Phe and protein. In particular, patients with classical (severe) PKU exhibit higher blood Phe concentrations, require the strictest diets, and are least likely to respond to Kuvan (sapropterin dihydrochloride), the only currently approved product for PKU. Thus, an unmet medical need exists for the majority of adult PKU patients. The blood Phe reductions demonstrated in the submitted clinical trials of PALYNZIQ observed in association with an unrestricted diet represent a major therapeutic advance in the treatment of adult PKU patients.

PALYNZIQ is associated with severe immune-mediated adverse reactions, including hypersensitivity reactions, anaphylaxis, generalized skin reactions, and arthralgias. Product labeling will include a Boxed Warning regarding anaphylaxis, a maximum recommended target dose of 40 mg/day, and criteria for treatment discontinuation for lack of efficacy. Labeling will also include a Medication Guide to inform patients about the identified risks. Further, a REMS with ETASU will ensure that prescribers and patients are aware of the risk of anaphylaxis, and ensure that safe use requirements are implemented, including that auto-injectable epinephrine is prescribed to all patients receiving PALYNZIQ and is readily available at all times.

The long-term safety of PALYNZIQ will be assessed post-approval via a prospective longitudinal observational study that will evaluate the rates of severe immune-mediated adverse reactions and will assess immunologic and inflammatory responses (e.g., anti-drug antibody titers, complement and CIC concentrations) and their effects on major organs such the kidneys. A postmarketing clinical trial will be required to assess the effects of a co-administered immune tolerance induction regimen on immune responses to PALYNZIQ and the potential for such a regimen to reduce the risk of severe immune-mediated adverse reactions.

A post-marketing prospective observational study will be implemented to collect long-term safety data in pregnant women treated with PALYNZIQ and outcomes in their offspring. An additional nonclinical study in pregnant rabbits given a diet devoid of Phe will be conducted to further explore the underlying mechanism for the observed fetal malformations in pregnant rabbits treated with pegvaliase.

16 Appendices

16.1 References

References are included in footnotes in the appropriate section.

16.2 Financial Disclosure

Covered Clinical Study (Name and/or Number): PAL-002, PAL-004, 165-205, PAL-003, 165-301, 165-302, 165-303

Was a list of clinical investigators provided:	Yes ☒	No ☐ (Request list from Applicant)
Total number of investigators identified: 300		
Number of investigators who are Sponsor employees (including both full-time and part-time employees): 0		
Number of investigators with disclosable financial interests/arrangements (Form FDA 3455): 3		
If there are investigators with disclosable financial interests/arrangements, identify the number of investigators with interests/arrangements in each category (as defined in 21 CFR 54.2(a), (b), (c) and (f)): Compensation to the investigator for conducting the study where the value could be influenced by the outcome of the study: 0 Significant payments of other sorts: 3 Proprietary interest in the product tested held by investigator: 0 Significant equity interest held by investigator in Sponsor of covered study: 0		
Is an attachment provided with details of the disclosable financial interests/arrangements:	Yes ☒	No ☐ (Request details from Applicant)
Is a description of the steps taken to minimize potential bias provided:	Yes ☒	No ☐ (Request information from Applicant)
Number of investigators with certification of due diligence (Form FDA 3454, box 3) Up-to-date financial disclosure information from 52 investigators who participated in the covered		

studies was not obtainable despite BioMarin's due diligence efforts in attempting to obtain the information. Financial disclosure information was obtained from the 52 investigators prior to their participation, and no disclosable financial interests or arrangements were reported at that time. The reason this information could not be obtained was because they were no longer at the site.		
Is an attachment provided with the reason:	Yes ☒	No ☐ (Request explanation from Applicant)

16.3 Nonclinical Pharmacology/Toxicology

See separate primary review (Reference ID: 4230623) and Section 5 above.

16.4 OCP Appendices (Technical documents supporting OCP recommendations)

See separate Clinical Pharmacology Memorandum to File (finalized May 22, 2018; Reference ID: 4266449).

16.5 Additional Clinical Outcome Assessment Analyses

Trial 165-303 was a phase 3 substudy of Trial 165-302 in which executive function as measured by selected tasks from the Cambridge Neuropsychological Test Automated Battery (CANTAB) was assessed in adults who participated in Trial 165-302. Executive function was assessed using three tasks from the CANTAB tool (Rapid Visual Processing [RVP], Spatial Working Memory [SWM], and Stop Signal Task [SST]). CANTAB data from 9 treated patients in Trial 165-302 part 2 were submitted for review. The Applicant noted in the Trial 165-303 CSR synopsis (page 7) that the results of the 3 primary efficacy endpoints of Trial 165-303 (data while on treatment in Trial 165-302 Part 2) numerically favored the pooled active groups. The review team's assessment of this submitted data concluded that there were no clear trends for treatment benefit and no clinically significant differences between the groups. In addition, the observed changes in the primary endpoints were not statistically significant. As such, this data was not considered in the assessment of efficacy for pegvaliase.

16.6 Patient Narratives

Increased blood CPK (6 SAEs in 5 patients)

Subject (b) (6) (trial 165-205)

24 year old male with an elevated CPK to 2449 U/L (ULN:235). "The subject had reportedly performed strenuous exercise for two consecutive days prior to the laboratory assessment. No treatment for the event was reported. Study drug was interrupted as a result of the event." 5 days later the event was resolved with CPK level of 210 U/L. The event did no recur when treatment resumed.

Subject (b) (6) (trial 165-301)

40 year old male who stopped exercising due to arthralgia after starting drug treatment. The subject started exercising again and two days later CPK level was 1195 U/L with elevated ALT and AST, normal bilirubin. He "reported starting a new exercise regimen prior to the most recent clinic visit. No treatment for the event was reported." About 3 weeks later, repeat CPK, ALT, and AST had all normalized. No recurrence.

Subject (b) (6) (2 events, trial 165-301)

28 year old male with CPK 9088 U/L (ULN 198) and elevated AST 352 U/L and ALT 189 U/L. The subject reported "that he had begun exercising much more frequently (including weight resistance training)" during the past 1-2 months. No treatment was reported. Repeat labs 2 days later showed improvement of CPK to 704 U/L with AST and ALT also trending down. At the study completion CPK normalized to 41 U/L. AST also normalized and ALT was still elevated at 75 U/L.

The subject started part 2 of study 302 with a normal CPK. At the end of part 2, the subject's CPK was 3661 U/L. He also complained of chest heaviness/tightness and shortness of breath. He was newly diagnosed with incomplete right bundle branch block. Repeat CPK was elevated to 3515 U/L. "The subject reported that he had begun exercising more frequently; he had been going to the gym regularly for about a month. Each workout was reported to last for 1.5 hours, including isolated muscle group workouts followed by cardiovascular training and a 30 minute muscle massage." The most recent workout was 4 days prior to the elevated CPK. Repeat CPK was significantly improved 1 day later and normalized 1 week later. No treatment for the event was reported.

Subject (b) (6) (trial 165-302)

39 year old male who started the T-25 exercise program and 3 days later had a CPK to 1030 U/L. No treatment for the event was reported. Repeat CPK 4 days later was normal. For the remainder of study 302, "the subject's CPK levels remained largely within normal range with occasional elevations not more than 2x ULN."

Subject (b) (6) (trial 165-302)

21 year old male with 2 mildly elevated CPK levels in study 301 (430 U/L, 214 U/L). In study 302, he had multiple occasions of mildly elevated blood CPK. "The subject reportedly ran more than 10 miles as part of his marathon training. The next day…his blood CPK was 3497 U/L, AST and ALT were within normal limits. He was reportedly asymptomatic, and no treatment for the event was reported." Normal repeat CPK about 2 weeks later. "The subject was reportedly active in marathon running during the studies."

16.7 Other Information

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Table 45: Schedule of Assessments - Trial 165-301

Table 8.5.1.1: Schedule of Events

Event or Assessment [a]	Screening	Induction		Non-Clinic Visits	Titration/ Maintenance	Study Completion Visit/ Early Termination Visit [c]	Hypersensitivity Reaction Visit [d]	Follow-up [e]
		Weeks 1, 2, 3	Week 4		Every 4 Weeks until 20 or 40 mg/day (Weeks 8, 12, 16, 20, 24, 28, 32) [b]			
	Days -28 to 0	Days 1, 8 (±1 day), 15 (±1 day)	Day 22 (±3 days)		Days 50, 78, 106, 134, 162, 190, 218 (±3 days)			
Informed consent	X							
Self-administration training	X	X (Days 1 and 8 only)						
Study drug observer training [f]	X							
Demographics	X							
Medical history, including allergy history	X							
PKU history [g]		X (Day 1 only)						
HIV test	X							
Hepatitis B and C test	X							
12-lead ECG	X					X		
Physical examination	X				X	X	X	
Vital signs	X	X (Day 1 only)	X		X	X	X	X
Weight and height	X				X (weight only)	X (weight only)		
Clinical laboratory tests (chemistry, hematology, urinalysis)	X		X		X	X	X	X
C-reactive protein	X				X	X	X	
Erythrocyte sedimentation rate [h]	X				X	X		

Event or Assessment [a]	Screening	Induction		Non-Clinic Visits	Titration/ Maintenance	Study Completion Visit/ Early Termination Visit [c]	Hypersensitivity Reaction Visit [d]	Follow-up [e]
		Weeks 1, 2, 3	Week 4		Every 4 Weeks until 20 or 40 mg/day (Weeks 8, 12, 16, 20, 24, 28, 32) [b]			
	Days -28 to 0	Days 1, 8 (±1 day), 15 (±1 day)	Day 22 (±3 days)		Days 50, 78, 106, 134, 162, 190, 218 (±3 days)			
Serum cortisol [i]		X (Day 1 only)				X		X
Complement panel (C3, C4)		X (Day 1 only)	X		X	X	X	
Tryptase		X (Day 1 only)					X	
Urine albumin/ creatinine ratio	X		X		X	X	X	
Urinalysis for N-methyl histamine							X	
Urine pregnancy test (if applicable) [j]	X	X (Day 1 only)	X		X	X		X
3-day diet diary [k]		X (Day 1 only)	X		X	X		X
Plasma PK [l]		X (Day 1 only)	X		X	X		
Immunogenicity assessments (antibodies) [m]		X (Days 1 and 15 only)	X		X	X	X (anti-rAvPAL-PEG IgE and rAv PAL IgE only)	X
Plasma Phe, tyrosine [n]	X	X (Days 1 and 15 only)	X		X	X		X
Adverse events [o]	X	X [o]	X	X	X	X	X	X
Telephone contact				X				
Concomitant medications	X	X [o]	X	X	X	X	X	X
ADHD-RS-Investigator rated [p]		X (Day 1 only)				X		
POMS-Subject Rated [p]		X (Day 1 only)				X		

Event or Assessment [a]	Screening	Induction		Non-Clinic Visits	Titration/ Maintenance	Study Completion Visit/ Early Termination Visit [c]	Hypersensitivity Reaction Visit [d]	Follow-up [e]
		Weeks 1, 2, 3	Week 4		Every 4 Weeks until 20 or 40 mg/day (Weeks 8, 12, 16, 20, 24, 28, 32) [b]			
	Days -28 to 0	Days 1, 8 (±1 day), 15 (±1 day)	Day 22 (±3 days)		Days 50, 78, 106, 134, 162, 190, 218 (±3 days)			
POMS-Observer Rated [p]		X (Day 1 only)				X		
Administer pegvaliase [q]		X	X		X	X [r]		

ACTH, adrenocorticotropic hormone; ADHD-RS, Attention Deficit Hyperactivity Disorder Rating Scale; C3, C4, complement components 3, 4; CTCAE, Common Terminology Criteria for Adverse Events; ECG, electrocardiogram; IgE, Immunoglobulin E; Phe, phenylalanine; PK, pharmacokinetics; POMS, Profile of Mood States.

All scheduled visits were performed in the clinic or by a home healthcare nurse.

[a] Event or assessment was performed pre-dose unless otherwise specified.

[b] Subjects were to complete the visits of the Titration Period every 4 weeks for as long as it took to achieve a dose of 20 mg/day or 40 mg/day. Subjects who reached their target maintenance dose of 20 or 40 mg/day were to continue to be seen in clinic every 4 weeks to be assessed until the Study Completion Visit. Once subjects had maintained a dose of 20 mg/day or 40 mg/day for at least 2 weeks after approximately Week 24, they were to complete the Study Completion Visit.

[c] The Study Completion Visit occurred at least 2 weeks after approximately Week 24 if subject reached a dose of 20 mg/day or 40 mg/day at approximately Week 24, or later for subjects who reached a dose of 20 mg/day or 40 mg/day after Week 24, up to Week 36. Self-administration of study drug continued throughout the week of the Study Completion Visit. Subjects who discontinued from the study early were to complete the Early Termination Visit within 4 weeks of the last dose of study drug. Subjects who discontinued from study drug early were asked to continue to complete the remaining study assessments until Week 36 as long as such continued participation did not detrimentally affect the health, safety, and welfare of the subject per investigator determination.

[d] Was to be performed if a subject had a CTCAE grade 3 or greater hypersensitivity reaction that was assessed by the investigator as related to treatment with pegvaliase. Was performed within 8-24 hours from event onset.

[e] Follow-up was performed at least 4 weeks after the Study Completion Visit (subjects who did not enroll in another available study where they could have received pegvaliase).

[f] Was performed for all observers identified (see Section 9.3.1). Was completed prior to administration of the first dose of study drug. Additional observers identified after screening were trained.

[g] As part of medical history, subjects were asked to provide information (per available data) regarding their PKU history to characterize the severity of subject's PKU and degree of historical disease control. These questions were developed by the sponsor based on input regarding clinical relevance to the patient population. Subjects were asked about their lifetime blood Phe levels (highest level and age when this occurred); how they categorized their metabolic control (excellent, good, poor, or none); and

at what age a low-Phe diet was discontinued (< 6 years old, 6 to ≤ 12 years old, 13 to ≤ 19 years old, 20 to 29 years old, or ≥ 30 years old). These questions were not validated for use amongst the PKU patient population and were only used in an exploratory manner.

[h] Performed by a local laboratory.

[i] Serum cortisol samples were taken before study drug administration in the morning before 10:00 am. A home healthcare nurse could have collected samples for subject convenience. If 2 results were low and abnormal, the subject was asked to perform additional sampling for plasma ACTH and a low-dose conventional ACTH test or the ACTH test method based on the clinical practice at the site.

[j] Performed locally. If urine pregnancy test was positive or equivocal, serum pregnancy test (central laboratory) was performed.

[k] Subjects were to complete the diet diary for three consecutive days prior to their next scheduled study visit.

[l] Plasma PK was collected pre-dose (within 1 hour of study administration) at the same time as sampling for blood Phe assessment.

[m] Performed on Day 1 of Week 1 and Day 15 of Week 2.

[n] Subjects were to fast for approximately 2.5 to 5 hours prior to plasma Phe assessment. Each plasma Phe assessment was to be performed at the same time of day. A central laboratory was used for blood Phe concentration analysis. A local laboratory could have been used for blood Phe concentrations when necessary at the Screening Visit only.

[o] Subjects were assessed for AEs and concomitant medications whenever they were assessed by study personnel. After written informed consent but before study treatment initiation, only SAEs associated with protocol-imposed interventions were recorded. After study drug initiation, all AEs and SAEs were recorded until 4 weeks after either the last administration of study drug or the Early Termination Visit.

[p] Administered at the same time of day. Administered ADHD-RS Investigator rated first followed by POMS.

[q] Self-administration of at least the first 2 doses of study drug was demonstrated and performed in the clinic. Thereafter, subjects were to self-administer study drug after demonstration of adequate ability and comprehension. Study drug was administered once weekly during the Induction Period. During the Titration Period, dosing frequency was increased to daily for doses > 40 mg/week. For non-clinic visits, subjects were asked about self-administration of study drug. Premedication requirements are outlined in Section 9.1.

[r] For Study Completion Visit only; not performed for Early Termination Visit.

Source: Applicant's submission, dated 6/30/2017, Module 5.3.5.2, CSR for study 301, pages 77 – 80.

Table 46: Schedule of Assessments-Trial 165-302, Part 2

Table 7.5.1.2: Schedule of Events, Part 2, Randomized, Double-Blind Discontinuation

Event or Assessment [a]	Weeks 1, 4, 8 Days 1, 28, 56 [b]	Nonclinic Visits Weeks 2, 3, 5, 6, 7
Physical examination	X	
Vital signs	X	
Weight	X	
Clinical laboratory tests	X	
C-reactive protein	X	
Erythrocyte sedimentation rate [c]	X	
Complements C_3 and C_4	X	
Urine albumin/creatinine ratio	X	
Urine pregnancy test [d]	X	
Diet diary [e]	X	
PK (plasma pegvaliase) [f]	X	
Plasma Phe, tyrosine [g]	X	
Immunogenicity assessments (antibodies)[h]	X	
Telephone contact		X
Adverse events [i]	X	X
Concomitant medication [i]	X	X
ADHD RS-IV (Investigator-Rated)	X	
POMS [j]	X	
Randomization [k]	Day 1 only	
Administer study drug [l]	X	X

ADHD RS-IV, Attention Deficit Hyperactivity Disorder Rating Scale IV (Investigator-Rated); eCRF, electronic Case Report Form; PK, pharmacokinetics; POMS, Profile of Mood States (Subject- and Observer-Rated).

All scheduled visits were performed in the study clinic; nonclinic visits were performed via telephone. Upon completion of Part 1, subjects who achieved a blood Phe reduction of ≥20% from baseline participated in Part 2.

[a] Events or assessments were predose unless otherwise specified.

[b] In Part 2, assessments scheduled for Weeks 1, 4, and 8 were performed on Day 1 (±1 day), Day 28 (±5 days), and within 5 days before Day 56, respectively.

[c] Was performed by a local laboratory.

[d] Was performed locally (if applicable). If urine pregnancy test was positive or equivocal, serum pregnancy test (central laboratory) was done (Section 9.1 of the study protocol; Appendix 16.1.1).

[e] Subjects were to record (at home) all food, beverages, special low-protein foods, and medical foods consumed during the three consecutive days preceding a study visit. No change in protein (natural foood and medical food) intake was allowed in Part 2.

[f] Blood was collected before study drug administration (predose).

[g] Blood was collected for plasma Phe analysis after fasting 2.5 to 5 hours.

[h] Immunogenicity assays included total anti-pegvaliase, anti-rAvPAL IgG, anti-rAvPAL IgM, anti-PEG IgM, anti-PEG IgG, and neutralizing antibodies.

[i] AEs and concomitant medications were assessed whenever a subject was assessed by study personnel. After written informed consent, all AEs and SAEs were recorded until 4 weeks after the last study drug dose or the Study Completion Visit/Early Termination Visit, whichever occurred last. If there was a skin reaction that lasted ≥ 14 days, the Skin Reaction eCRF could have been completed. A photograph of the skin reaction could have been taken by the subject or the site to help assess the event and could have been collected by the sponsor.

[j] ADHD RS-IV (Investigator-Rated) was administered first, followed by POMS (Subject- and Observer-Rated), at the same time of day throughout the study.

[k] Randomization occurred Part 2, Week 1 for all subjects.

[l] Subjects self administered study drug daily. Subjects could have been premedicated with H1 antagonist, H2 antagonist, and non-steroidal anti-inflammatory medication (NSAIDs) approximately 2 to 3 hours prior to study drug per investigator determination as outlined in Section 9.1 of the study protocol (Appendix 16.1.1). NSAIDs should have been given with food and could have been omitted if not tolerated. For Nonclinic Visits, subjects were asked about study drug self administration.

Source: Applicant's submission, dated 6/30/2017, Module 5.3.5.1 CSR for study 302, pages 127 – 128.

Table 47: Patient Disposition- Trials 165-301 and 165-302

End of Study 301 Status			Screened for Study 301			End of Study 302 Screening			End of Study 302 Part 1			End of Study 302 Part 2					Enroll in Part 4
	20 mg (N=131)	40 mg (N=130)		20 mg (N=112)	40 mg (N=103)		20 mg (N=112)	40 mg (N=103)		20 mg (N=86)	40 mg (N=78)		20 mg (N=34)	20 mg P (N=15)	40 mg (N=32)	P (N=14)	N=202
Completed Study 301 , reached the maintenance dose, and screened for Study 302	80 (61.1%)	72 (55.4%)	Screened for part 1	80+6	72+6	Entered Study 302 Part 1	80+6	72+6	Entered Study 302 Part 2 and reached >=20% reduction	39+4 (50.0%)	38+5 (55.1%)	Entered Study 302 Part 3	25+3 (82.4%)	13+1 (93.3%)	22+3 (78.1%)	13+0 (92.9%	80 (39.6%)
												Entered Study 302 Part 4 due to AE	1+0 (2.9%)	0	1+2 (9.4%)	1+0 (7.1%)	5 (2.5%)
												Early termination from Study - withdrawal by	0	0	1+0 (3.1%)	0	
									Entered Part 2 without reaching 20% reduction	6+0 (7%)	3+0 (3.8%)	Entered Study 302 Part 3	5+0 (14.7%)	1+0 (6.7%)	3+0 (9.7%)	0	9 (4.5%)
									Entered Study Part 4 without reaching >= 20% reduction	26+0 (30.2%)	12+0 (15.4%)						37 (18.3%)
									Entered Part 4 due to early closure of Study 302 Part 2	4 (4.7%)	6 (7.7%)						10 (5.0%)
									Entered Study 302 Part 4 for other reasons	0+2 (2.3%)	6+1 (9.0%)						10 (5.0%)
									Early Study discontinuation in 165-302 Part 1	5+0 (5.8%)	7+0 (9.0%)						
									Lost to follow-up	1	3 (3.8%)						
									Adverse event	3 (3.5%)	1 (1.3%)						
									Physician decision	0	2 (2.6%)						
									Withdrawal by subject	1 (1.2%)	1 (1.3%)						
Completed Study 301, screened for Study 302, and enrolled in 302 Part 4 directly	26 (19.8%)	25 (19.2%)	Screened for Part 4	26	25	Entered Part 4 directly	26	25									51 (25.2%)
maintenance dose not reached	6 (4.6%)	10															
early closure of Study 302 Part 2	20 (15.3%)	15															
Completed Study 301 and not screen in Study 302	5 (3.8%)	5 (3.8%)															
Completed treatment	3 (2.3%)	1 (0.8%)															
Early Study Drug Discontinue	2 (1.5%)	4 (3.1%)	AE	2 (1.5%)	3 (2.3%)												
			Pregnancy	0	1 (0.8%)												
Early Study Discontinuation	20 (15.3%)	28 (21.5%)	Early Study Drug Discontinue	22 (16.8%)	32 (24.6%)												
Adverse Event	8 (6.1%)	9 (6.9%)	AE	8 (6.1%)	9 (6.9%)												
Death	1 (0.8%)	0	AE	1 (0.8%)	0												
Lost to follow-up (LTFU)	1 (0.8%)	1 (0.8%)	LTFU	0	1 (0.8%)												
			AE	1 (0.8%)	0												
Physician decision (PhyD)	3 (2.3%)	2 (1.5%)	PhyD	3 (2.3%)	1 (0.8%)												
			AE	0	1 (0.8%)												
Pregnancy	1 (0.8%)	0	Pregnancy	1 (0.8%)	0												
Withdrawal by subject (Withdrawal)	6 (4.6%)	11 (9.2%)	Withdrawal	5 (3.8%)	9 (6.9%)												
			AE	1 (0.8%)	2 (1.5%)												
Other	0	2 (1.5%)	Other	0	2 (1.5%)												
Protocol Deviation (PD)	0	2 (1.5%)	PD	0	2 (1.5%)												
Study terminated by sponsor	0	1 (0.8%)	AE	0	1 (0.8%)												

Note: (1) The percentage is out of the "N" in the column header; (2) N=131, 130 are number of subjects enrolled in study 301; N=112, 103 are number of subjects screened for study 302; N=86, 78 are number of subjects enrolled in study 302 Part 1; N=34, 15, 32, 14 are number of subjects enrolled in study 302 Part 2; N=202 is the number of subjects enrolled in study 302 Part 4. (3) Numbers after "+" are the number of patients came from Phase 2 studies.

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

JULIE G BEITZ
05/24/2018

My review is complete and has been added to the multidisciplinary review and evaluation document. My review is based on the information currently in the administrative record. If I must review information that is subsequently added to the administrative record, I will update my part of the multidisciplinary review and evaluation document accordingly.

Attached below is "Section 16.4 OCP Appendices" to the BLA761079 Multi-Disciplinary Review and Evaluation. The OCP Appendices include technical documents that support OCP recommendations in the Clinical Pharmacology section of the Multi-Disciplinary Review and Evaluation. The Multi-Disciplinary Review and Evaluation will be uploaded to DARRTS separately.

Office of Clinical Pharmacology (OCP) Appendices

NDA or BLA Number	BLA 761079
Link to EDR	\\CDSESUB1\evsprod\BLA761079\761079.enx
Submission Date	6/30/17
Submission Type	Priority
Brand Name	Palynziq
Generic Name	Pegvaliase
Dosage Form and Strength	Single-dose prefilled syringes (PFS): 2.5 mg/0.5 mL 10 mg/0.5 mL 20 mg/1 mL
Route of Administration	Subcutaneous (SC) injection
Proposed Indication	To reduce blood phenylalanine (Phe) in adult patients with phenylketonuria (PKU) who have uncontrolled blood phenylalanine levels > 600 µmol/L on existing management
Applicant	BioMarin
Associated IND	IND 076269
Clinical Pharmacology Reviewer	Christine Yuen-Yi Hon, Pharm.D.
Clinical Pharmacology Team Leader	Jie Wang, Ph.D.
Pharmacometrics Reviewer	Youwei Bi, Ph.D.
Pharmacometrics Team Leader	Kevin Krudys, Ph.D.
OCP Final Signatory	Shiew Mei Huang, Ph.D. Acting Division Director (DCP3)
OCP Division	Division of Clinical Pharmacology 3 (DCP3)
OND Division	CDER/ODEIII/DGIEP

Table of Contents

1 OCP APPENDICES

1.1 Summary of Bioanalytical Method Validation and Performance

1.1.1 Bioanalytical methods for the determination of pegvaliase concentrations in human plasma

The Applicant developed three quantitative sandwich enzyme-linked immunosorbent assays (ELISA) for the measurement of pegvaliase concentrations in human plasma. The ELISA methods used the rabbit monoclonal anti-PEG IgG and the rabbit polyclonal anti-rAvPAL IgG as capture/detection agents. All methods used a biotinylated detection reagent, followed by streptavidin-conjugated horseradish peroxidase and tetramethyl benzidine (TMB) substrate for colorimetric development. Pharmacokinetic (PK) samples were acidified and neutralized to dissociate ADA from pegvaliase before adding to the assay plate. Hence, the ELISA assay measures total plasma pegvaliase concentrations including ADA-bound pegvaliase and ADA unbound (or "free") pegvaliase. See information below for more information.

The reagents and assay performances of the ELISA assays are summarized in **Table 1**. The validation parameters and performance of the ELISA assay used for phase 3 trials are further summarized in **Table** 2**.**

Table 1 Summary of reagents and assay performance of ELISA assays in pegvaliase clinical trials

Studies Supported	Phase 1	Phase 2	Phase 3
Acid Dissociation	0.1 M Glycine pH 2.7	0.1 M Glycine pH 2.7	40 mM Acetic Acid
Neutralization	0.5 M Tris, pH 8.5	0.5 M Tris pH 8.5	300 mM Tris-HCl / 2mM CTAB[a] pH 7.5
Capture Reagent	Rabbit polyclonal anti-rAvPAL IgG	Rabbit polyclonal anti-rAvPAL IgG	Rabbit monoclonal anti-PEG IgG
Detection Reagent	Rabbit monoclonal anti-PEG IgG	Rabbit monoclonal anti-PEG IgG	Rabbit polyclonal anti-rAvPAL IgG
Minimum Required Dilution	1:50	1:20	1:50
Lower Limit of Quantitation	25 ng/mL (neat plasma)	4 ng/mL (neat plasma)	75 ng/mL (neat plasma)
Precision and Accuracy (%CV[b] and %RE[c])	<40%	<30%	<20%
ADA Interference	anti-rAvPAL IgG: >0.4 µg/mL	anti-rAvPAL IgG: 0.2 µg/mL	anti-rAvPAL IgG: 25 µg/mL
	Anti-PEG: N.D.[d]	anti-PEG IgG: 2 µg/mL	anti-PEG IgG: 100 µg/mL

[a] CTAB: Hexadecyltrimethylammonium Bromide

[b] CV: coefficient of variation

[c] RE: relative error

[d] N.D.: not determined

(Source of data: Table 2.7.1.1.1.1.1, Module 2.7.1 Summary of Biopharmaceutic Studies and Associated Analytical Methods)

Table 2. Summary of assay validation parameters of the ELISA assay for measurement of pegvaliase concentrations in human plasma in Phase 3 trials

Assay Parameter	Acceptance Criteria	Validation Results
Intra-Assay Accuracy and Precision (QCs, LOQs)	(Pooled) |%RE| ≤ 25.0%, 25.0% at LOQs (Pooled) %CV ≤ 20.0%, 25.0% at LOQs (Intra-run) %TE ≤ 35.0%, 35.0% at LOQs	|%RE| ≤ 7.1%, 3.0% at LOQs %CV ≤ 7.8%, 13.4% at LOQs %TE ≤ 14.9%, 16.4% at LOQs
Inter-Assay Accuracy and Precision (QCs, LOQs)	|%RE| ≤ 25.0%, 25.0% at LOQs %CV ≤ 20.0%, 25.0% at LOQs %TE ≤ 35.0%, 35.0% at LOQs	|%RE| ≤ 7.1%, 3.0% at LOQs %CV ≤ 15.7%, 21.0% at LOQs %TE ≤ 22.8%, 24.0% at LOQs
Standard Curve	Mean 5PL curve fit $R^2 \geq 0.98$ Absolute %RE ≤ 20.0%, 25.0% at LOQs %CV ≤ 20.0%, 25.0% at LOQs	Mean 5PL curve fit $R^2 \geq 0.996$ Absolute %RE ≤ 2.9%, ≤ 3.6% at LOQs %CV ≤ 7.9%, ≤ 10.1% at LOQs
Selectivity	|%RE| ≤ 25.0%, %CV ≤ 20.0% for 80% of spiked individual samples at each level 100% of non-spiked samples BLQ	9 / 10 of high spiked samples met criteria 9 / 10 of low spiked samples met criteria 10 / 10 of non-spiked samples were BLQ No interference with lipemic samples observed Potential interference for highly hemolytic samples
Specificity	|% RE| ≤ 25.0%, % CV ≤ 20.0% in the presence of free rAvPAL, PEG, anti-rAvPAL IgG or anti-PEG IgG Non-specific analytes in Matrix = BLQ	rAvPAL: tolerant up to 78 ng/mL; %CV ≤ 13.8, mean |%RE| ≤ 9.7 PEG: tolerant up to 8 ug/mL; %CV ≤ 20.6%, mean |%RE| ≤ 22% Anti-rAvPAL: tolerance up to 2.5 µg/mL; %CV ≤ 6.6%, mean |%RE| ≤ 24.7% Anti-PEG: tolerance up to 50 µg/mL; %CV ≤ 5.8%, mean |%RE| ≤ 11.0% Non-specific analyte in Blank Matrix: 100% tested BLQ
Robustness	Plate Acceptance: |%RE| ≤ 25.0% and %CV ≤ 20.0% for ≥ 2/3 HQC and LQC Incubation Times Criteria: ≥ 2/3 Plates must pass QC acceptance criteria	3/3 Minimum incubation time plates passed acceptance criteria 3/3 Standard incubation time plates passed acceptance criteria 3/3 Maximum incubation time plates passed acceptance criteria
Sample Stability	Plate Acceptance: |%RE| ≤ 25.0% and %CV ≤ 20.0% for ≥ 2/3 HQC and LQC Stability Acceptance: |%RE| ≤ 25.0% and %CV ≤ 20.0% for ≥ 2/3 Stability QCs	HQC and LQC met acceptance criteria after up to five hours at room temperature and up to 48 hours at 2-8°C HQC and LQC met acceptance criteria after up to 5 F/T cycles

Assay Parameter	Acceptance Criteria	Validation Results
Dilution Linearity	|% RE| within ROQ ≤ 25.0%, %CV ≤ 20.0% across dilution series	|% RE| within ROQ ≤ 12.0% %CV ≤ 18.8% across dilution series
Interim ISR[a]	|%Difference| ≤ 30.0% for ≥ 67% of the samples	65.2% of ISR study samples demonstrated |%Difference| ≤ 30.0[a]
LTS	At least 4 out of 6 stability QCs (3 HQC and 3 LQC) must have %CVs and |%RE| ≤ 25.0% with at least 50% passing at each level	The QCs met the LTS acceptance criteria for the following storage condition and maximum duration: -60 to -80°C for 95 weeks (664 days)

[a] ISR results are documented in BAS-GR-17-029, Incurred Sample Reanalysis Report for the Detection of BMN165 in Human Plasma Using a Ligand Binding Assay. An ISR investigation (BAS-GR-17-033) led to an assignable cause for 35 samples that impacted the overall ISR passing rate and caused it to drop slightly below the guidance criteria of 67%; excluding these samples would result in 69.1% of samples with ≤ |30% difference| between original and ISR results.

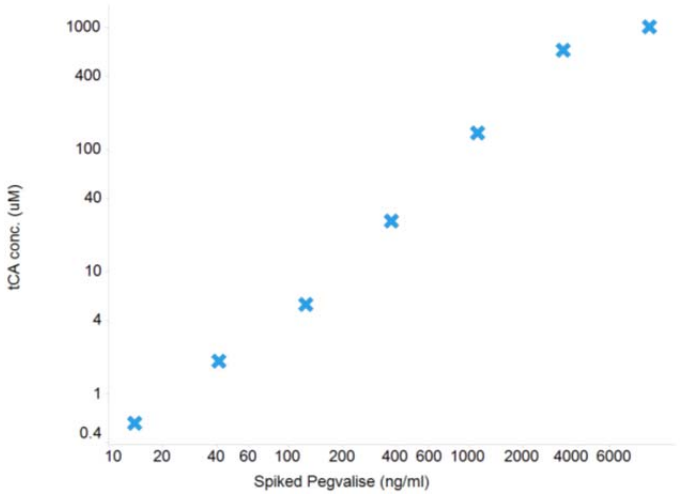
(Source of data: Validation report BMN165-13-022I; Table 2.7.1.1.1.1.2 Summary of Biopharmaceutic Studies and Associated Analytical Methods)

1.1.1.1 Impact of acid dissociation on the performance of the ELISA PK Assay

Using a representative subset of plasma samples from study 165-301, the Applicant compared the performance of the validated Phase 3 PK assay with a modified PK method without the acid pretreatment and neutralization steps. The modified PK method presumably measures free (not ADA-bound) pegvaliase. The Applicant also assessed the activity of the ADA-bound pegvaliase complexes using a mass spectrometry (MS)-based PAL activity assay developed as a component of the pegvaliase-neutralizing antibody (NAb) assay (Validation BMN165-13-053/ (b) (4) -181171 and BAS-GP-16-057).

The activity assay measures the concentration of the enzymatic product tCA after incubation of pegvaliase with a fixed Phe substrate concentration, and the tCA concentration generated is used to assess the relative level of pegvaliase activity in ADA-bound pegvaliase complexes isolated from clinical samples by the protein A&G-sepharose resin. The standard curve showing the production of tCA from pegvaliase is depicted in **Figure 1**.

Figure 1. tCA production curve to reflect pegvaliase activity

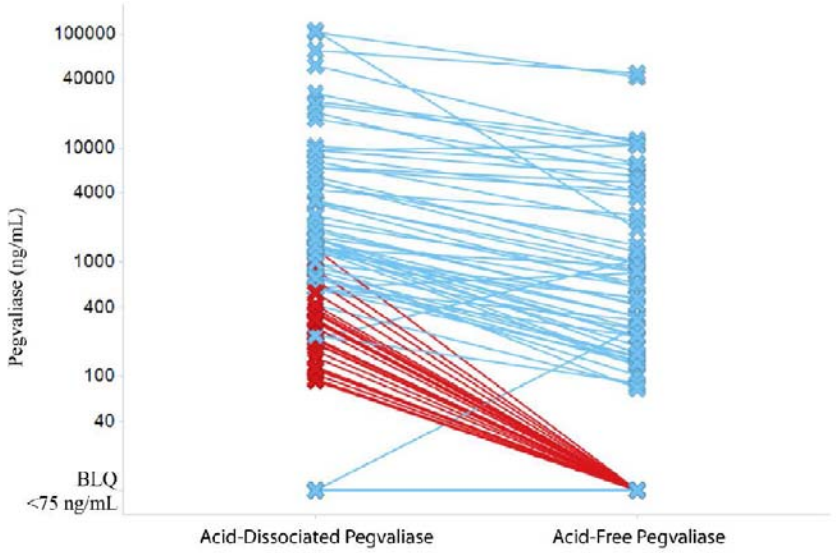


Pegvaliase conc. (ng/mL)	0	13.7	41.2	123.5	370	1111	3333	10000
tCA conc. (µM)	-	0.579	1.86	5.39	26.0	137	648	1010

(Source of data: Figure 6.1.2.1 of Investigation of the Utility of Acid Dissociation in the Pegvaliase Pharmacokinetic ELISA Report; BAS-GR-16-057)

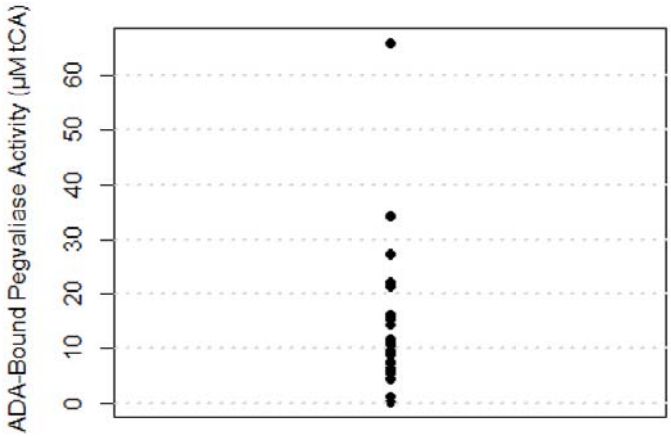
A total of 164 PK samples from Study 165-301 were assessed for pegvaliase concentrations using the PK assay both with and without acid dissociation. The majority of these clinical samples contained ADA, and pegvaliase concentrations determined without acid dissociation were lower than concentrations measured after acid dissociation (**Figure 2**). One sample (0.6%) had detectable pegvaliase without acid dissociation but was below the limit of quantitation (BLQ) with acid dissociation. Twenty-five of the 164 (15.2%) samples changed from quantifiable in the re-tested acid dissociation method to BLQ when the acid step was removed (red lines and symbols in **Figure 2**). The Applicant further measured the ADA-bound pegvaliase activity of these 25 samples; 24/25 (96%) of the samples that were below BLQ in acid-free assay had ADA-bound pegvaliase activity > 0.250 µM tCA (**Figure 3**).

Figure 2. Comparison of pegvaliase concentrations measured using assays with or without acid dissociation pre-treatment of PK samples



(Source of data: Figure 6.4.1 of Investigation of the Utility of Acid Dissociation in the Pegvaliase Pharmacokinetic ELISA Report; BAS-GR-16-057)

Figure 3. ADA-bound pegvaliase activity in samples below BLQ in acid-free PK assay but quantifiable in acid-dissociated PK assay



Out of 164 selected samples, 25 samples were BLQ in the acid-free assay but quantifiable in re-tested acid-dissociation PK assay. ADA-bound activity was tested in these samples. Each point represent a sample.

(Source of data: Figure 6.4.2 of Investigation of the Utility of Acid Dissociation in the Pegvaliase Pharmacokinetic ELISA Report; BAS-GR-16-057)

Reviewer's Comments

The Applicant demonstrated that ADA-bound pegvaliase complexes from the 25 PK samples with BLQ concentration in the acid-free assay were enzymatically active. However, the

pegvaliase concentrations (in ng/mL) back calculated using the enzyme activity were lower than the pegvaliase concentrations obtained by the assay with the acid dissociation step. This indicated that pegvaliase concentrations measured with the acid dissociation step could overestimate the enzymatically active pegvaliase concentrations. On the other hand, pegvaliase concentrations measured without acid dissociation could underestimate the enzymatically active pegvaliase concentrations. Additionally, due to inter-subject variability in ADA responses and the dynamic nature of ADA responses over time, it is not feasible to estimate the fraction of ADA-bound pegvaliase that remains enzymatically active for each PK sample. These limitations suggest that interpretation of the PK data based on total pegvaliase concentrations and exploration of exposure-response relationships based on PK data need to be conducted with caution.

1.1.2 Bioanalytical method for the measurement of Phenylalanine (Phe) concentrations in human plasma

Plasma Phe and tyrosine (Tyr) concentrations were measured by an ion exchange (IE) chromatography method using the amino acid analyzer Biochrom 20 plus or Biochrom 30. The assay was validated by the (b) (4) and was used for analyzing all samples from pegvaliase clinical trials. **Table 3** presents the summary of the assay performance (validation BG019).

Table 3. Summary of assay performance for the bioanalytical method measuring Phe concentrations in human plasma

Assay Parameter	Performance	
	Phe	Tyr
Concentration linearity	Linear up to 3750 µmol/L	Linear up to 3750 µmol/L
Precision (% Coefficient of variation)		
Between Run	3.5 %	3.7%
Within Run	10.5 %	7.0 %
Sensitivity	5 µmol/L	5 µmol/L
Reportable Range	5 – 3750 µmol/L	5 – 3750 µmol/L
Reference Range		
Newborn (< 1 year)	30 – 100 µmol/L	30 – 140 µmol/L
Adult (> 1 year)	30 – 80 µmol/L	30 – 120 µmol/L

(Source of data: Validation report RD-VAL-140 Rev.1; Table 2.7.1.1.1.2.1, Summary of Biopharmaceutic Studies and Associated Analytical Methods)

1.1.3 Immunogenicity assays: bioanalytical methods for testing anti-drug antibodies (ADA), neutralizing antibodies (NAb), and circulating immune complexes (CICs)

The Applicant used a panel of 10 different ADA assays and two CIC assays for assessment of pegvaliase immunogenicity and potential effects of anti-pegvaliase immune responses in clinical trials. Separate electrochemiluminesence assay (ECLA) methods were validated for testing anti-rAvPAL IgG, anti-rAvPAL IgM, anti- PEG IgG, anti-PEG IgM, and TAb (anti-pegvaliase IgG, IgM,

and IgA) to support phase 3 studies. A validated hybrid ligand-binding: liquid chromatography/tandem mass spectrometric assay was used to measure titers of NAb. Radioimmunoassays (RIAs) were developed to measure rAvPAL-specific IgE and pegvaliase-specific IgE in Phase 1 through 3 studies. Subsequently, a pre-clearing step was added to remove IgG and IgM, and anti-rAvPAL and anti-pegvaliase IgE assays were re-developed and validated using the ImmunoCAP 1000 platform to support and confirm Phase 3 IgE results.

Table 4 provides a summary of the sensitivity and drug tolerance of the immunogenicity assays used in the phase 3 studies. For reference, the trough pegvaliase concentrations in Study 165-302 Part 4 ranged from 75 to 132000 ng/mL. Refer to the Drug Product Quality Review/ Immunogenicity Review from Dr. Frederick Mills for details regarding the performance and validation of the immunogenicity assays.

Table 4. Summary of the sensitivity and drug tolerance of the Phase 3 immunogenicity assays

Assay	Validation Report	Limit of Detection	Drug Tolerance
Anti-rAvPAL IgG	BMN165-13-048	122 ng/mL	LPC: 100 ng/mL HPC: 2500 ng/mL
Anti-rAvPAL IgM	BMN165-13-050	42 ng/mL	LPC: 100 µg/mL HPC: 100 µg/mL
Anti-PEG IgG	BMN165-13-049	205 ng/mL	1 ng/mL
Anti-PEG IgM	BMN165-13-051	167 ng/mL	10 ng/mL
Anti-pegvaliase TAb	BMN165-13-052	1450 ng/mL for anti-PAL IgG 147 ng/mL for anti-PEG IgG	LPC: 100 ng/mL HPC: 100 µg/mL
NAb	BMN165-13-053	1490 ng/mL	LPC: 25 µg/mL HPC: 100 µg/mL
Anti-pegvaliase and anti-rAvPAL IgE by RAST	PS-2556-1VR	0.34 kU/L = 0.85 ng/mL	ND
Anti-pegvaliase IgE by ImmunoCAP	21120-4767	0.10 kU/L = 0.35 ng/mL	25 µg/mL
Anti-rAvPAL IgE by ImmunoCAP	21120-4768	0.10 kU/L = 0.24 ng/mL	25 µg/mL
Anti-pegvaliase IgG4	BMN165-16-055	18.3 ng/mL	LPC: 1 µg/mL HPC: 500 µg/mL
IgG-C3d CIC*	CVL072409	300 ng Eq/mL	ND
IgM-C3d CIC*	IM-VAL-011	1 µg Eq/mL	ND

LPC, low positive control; HPC, high positive control. *The upper limit of normal for IgG-C3d and IgM-C3d CIC concentrations in normal human serum is 36 µg Eq/mL and 7.6 µg Eq/mL, respectively.

Reviewer's Comments

For immunogenicity samples from Phase 1 and 2 studies, anti-rAvPAL IgG, anti-rAvPAL IgM, anti- PEG IgG, and anti-PEG IgM ADAs were analyzed by four separate enzyme-linked immunosorbent assays (ELIZA), whereas NAb were detected by a spectrophotometric enzymatic

assay. The RAST assay used for the detection of anti-rAvPAL and anti-pegvaliase IgE antibodies (PS-2556-1VR) in Phase 3 was also used to analyze clinical samples from Phase 2 studies.

1.1.3.1 The impact of pegvaliase concentration on the incidences and mean antibody titers of PEG IgG and PEG IgM

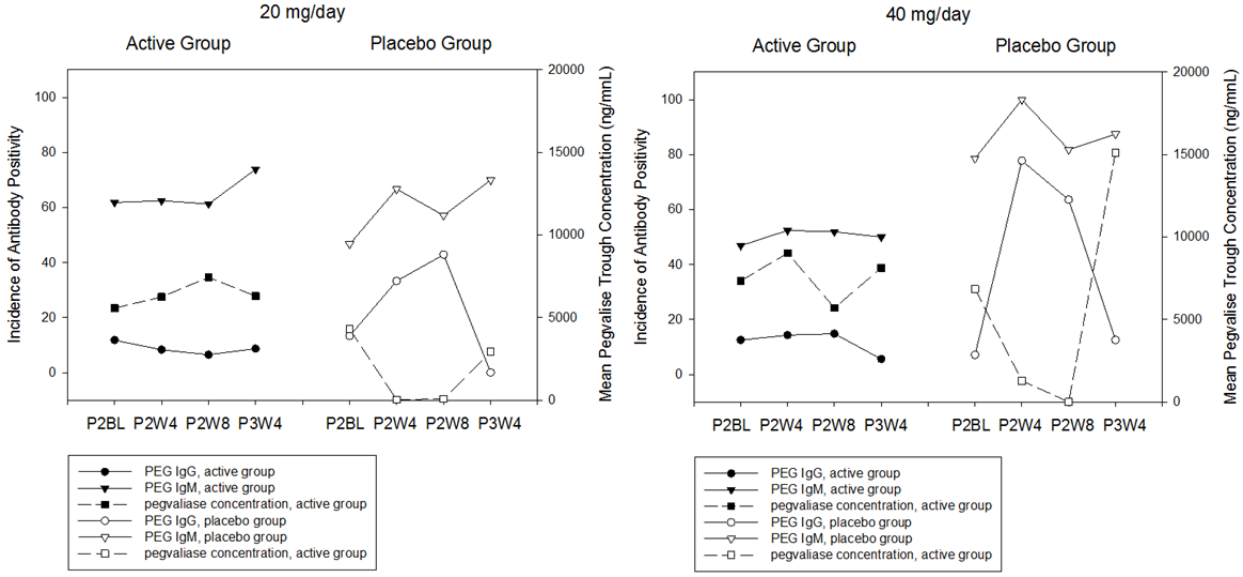
The two anti-PEG antibody assays had poor drug tolerance to enable detection of anti-PEG antibodies in the presence of pegvaliase. Specifically, the drug tolerance for the anti-PEG IgG and IgM assays was 1 ng/mL and 10 ng/mL of pegvaliase, respectively. Because the lower limit of quantitation (LLOQ) of the PK assay was 75 ng/mL, any measurable pegvaliase concentrations (i.e., ≥ 75 ng/mL) could interfere with the detection of anti-PEG antibodies.

The impact of pegvaliase concentrations on the incidences and mean antibody titers of anti-PEG antibodies is demonstrated during the randomized discontinued part of 165-302 (i.e., Part 2) and the re-introduction of pegvaliase administration in Part 3. For the active treatment groups (both 20 mg and 40 mg), there was slight variability in the incidences and mean antibody titers of anti-PEG IgG and IgM throughout Parts 2 and 3 (**Figure 4**). On the other hand, the incidence of PEG IgG increased from approximately 7% in Part 2 baseline to 77.8% in Part 2 Week 4, declined slightly to 63.6% in Part 2 Week 8, and then decreased to 12.5% in Part 3 Week 4 in the 40-mg placebo group. These changes in incidence paralleled the changes in the mean pegvaliase concentration, which decreased to 0 ng/mL in Part 2 Week 8 during the discontinuation period and increased to 15130 ng/mL in Part 3 Week 4. Mean anti-PEG IgG titer increased during drug discontinuation and decreased after pegvaliase reintroduction in a similar fashion; the titer changes followed the mean pegvaliase concentration changes but in an opposite direction. Although the magnitude of changes was smaller, similar changes were observed in the 20-mg placebo group.

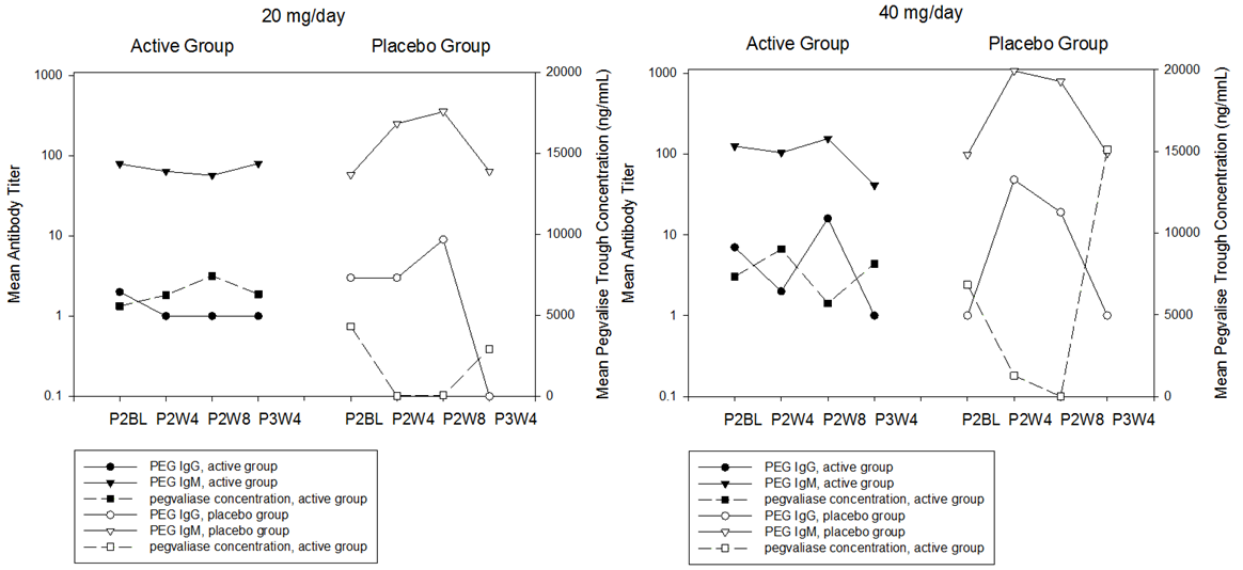
Likewise, the changes in the incidence and mean anti-PEG IgM titer were associated with mean pegvaliase concentration changes (**Figure 4**).

Figure 4. (a) Incidences of antibody positivity and (b) mean antibody titers for the 20 mg/day (left) and 40 mg/day (right) treatment groups in Study 165-302 Parts 2 and 3

(a) Immunogenicity incidences



(b) Mean antibody titers



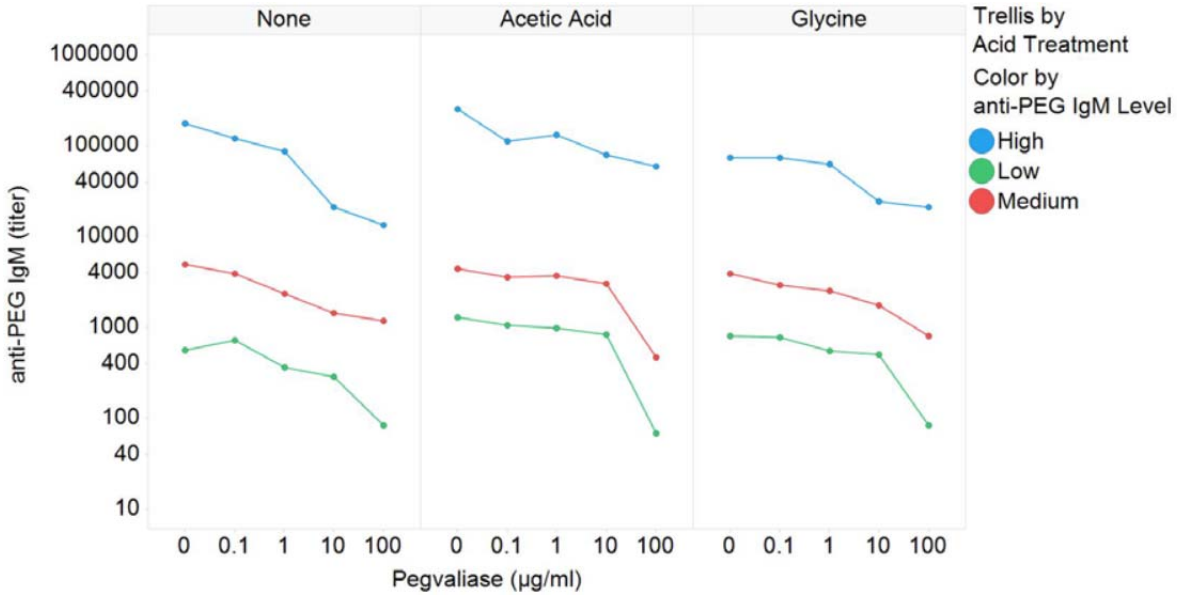
Abbreviations: P2BL, Part 2 Baseline; P2W4, Part 2 Week 4; P2W8, Part 2 Week 8; P3W4, Part 3 Week 4
Note: In 165-302, pegvaliase was discontinued in Part 2 from baseline to Week 8 and was reintroduced starting from Part 2 Week 8 into Part 3 in the placebo group only. Pegvaliase treatment was continued throughout Part 2 to Part 3 in the active treatment groups.

(Source of data: Reviewer's analysis based on data from Tables 14.2.7.2, 14.2.7.3, 14.3.6.70. 14.3.6.71, 14.3.6.75, and 14.3.6.76 of 162-302 CSR)

The Applicant evaluated the impact of pegvaliase concentration on anti-PEG ADA titers by adding free pegvaliase into pooled samples from 165-301 that had different levels (high, medium, and low) of each ADA analyte and no detectable pegvaliase concentration (i.e., < 75 ng/mL). The drug tolerance experiments were performed using the current anti-PEG ADA assays with or without pre-treatment with acetic acid or glycine.

The experimental results showed that acid dissociation improved the current anti-PEG IgM assay. Overall, higher anti-PEG IgM titer values were detected for all pooled samples, with several-fold increase in titer value for pooled samples with high PEG IgM titer (blue line and symbols in **Figure 5**). The effects of pegvaliase concentration on anti-PEG IgM titer were also minimized with acid dissociation for the medium and low titer samples, as evidenced by the smaller decline in titer value with increasing pegvaliase concentration. Thus, acid dissociation should be incorporated into the anti-PEG IgM ADA assay to allow better characterization of the association between anti-PEG IgM titer and the incidence of HAEs (see Section 1.4.9 for impact of immunogenicity on safety for more information).

Figure 5. Drug tolerance of the anti-PEG IgM titer assay



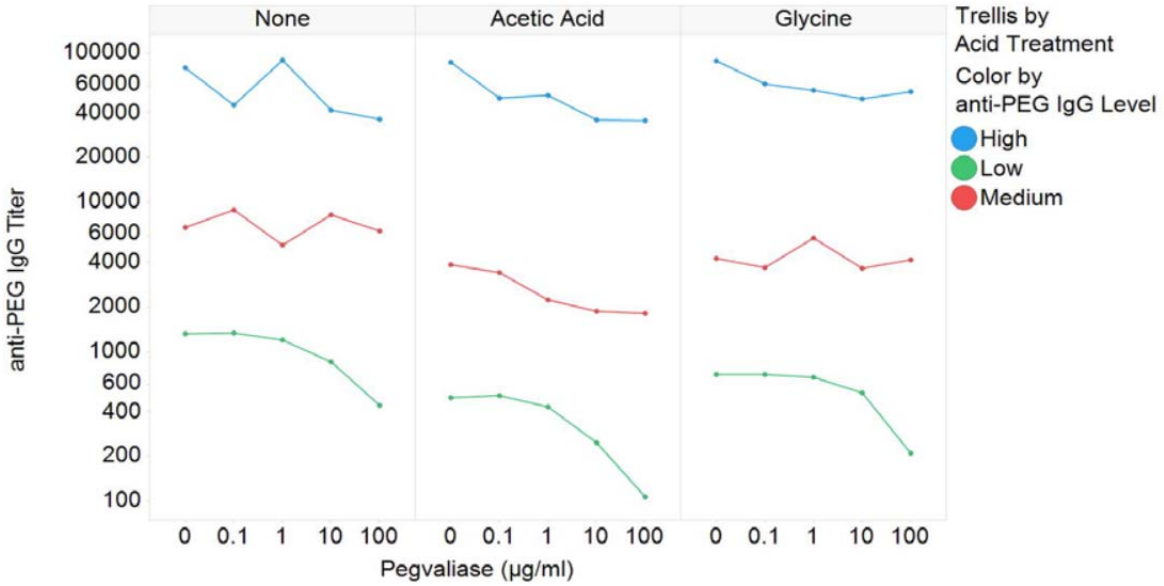
(Source of data: Figure 8.6.1 of BAS-GR-17-032 Investigation of Pegvaliase Anti-Drug Antibody Titer Assay Drug Tolerance)

On the other hand, acid dissociation reduced the sensitivity of the anti-PEG IgG ADA assay, as demonstrated by an overall lower anti-PEG IgG titer values with acid dissociation compared to the current assay (**Figure 6**). The detection of anti-PEG IgG titers in the presence of pegvaliase concentration was not improved as well. Glycine pre-treatment produced similar drug interference results as the current anti-PEG IgG ADA assay.

Nonetheless, improving the anti-PEG IgG ADA assay is important because a clear inverse relationship between anti-PEG IgG incidence and mean pegvaliase trough concentration was

observed during the randomized discontinuation period (i.e., 165-302 Part 2) followed by the reintroduction of pegvaliase in 165-302 Part 3 (**Figure 4** and text as described above). In addition, half of the samples was below a titer value of 90 (i.e., median anti-PEG IgG titer value in **Table 18**) and three quarters of the samples were below a titer value of 810 (75th percentile anti-PEG IgG titer value in Table 14.3.6.3.3 of 165-301 CSR) at Week 8 of 165-301. Considering the degree of titer decrease for the low titer pooled sample (green line in **Figure 6**), titer value for some if not all of the low titer samples would likely decrease and become undetectable when mean pegvaliase concentration increased from 61.3 ng/mL at Week 8 to 1974 ng/mL at Week 12 and thereafter (**Table 5**). These data suggest that the decline in anti-PEG IgG incidence from 95% at Week 8 to 65% in Week 12 was likely an artifact of drug interference (**Figure 7a**).

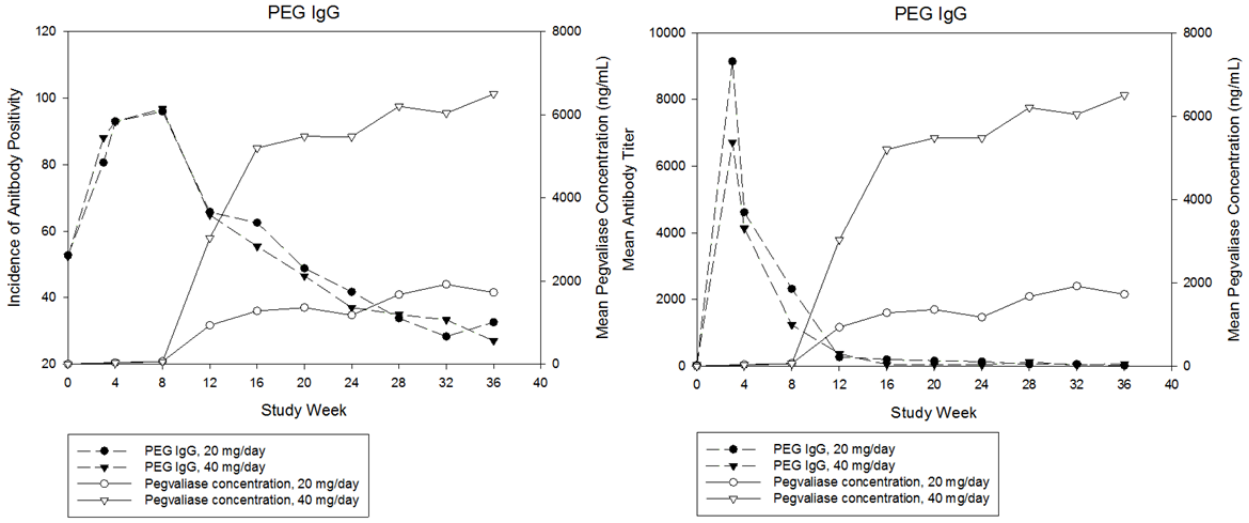
Figure 6. Drug tolerance of the anti-PEG IgG titer assay



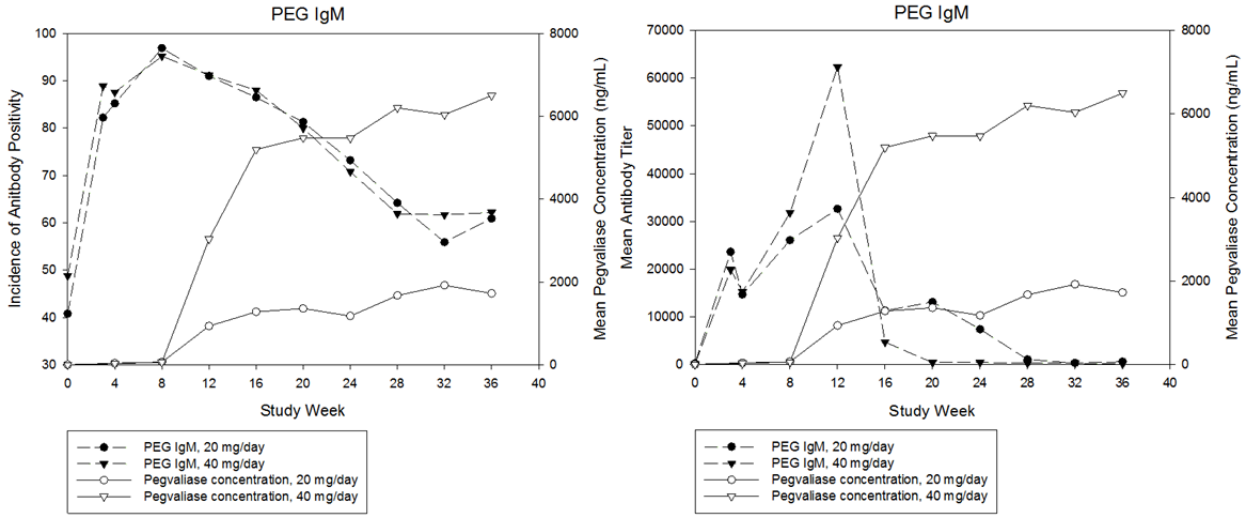
(Source of data: Figure 8.7.1 of BAS-GR-17-032 Investigation of Pegvaliase Anti-Drug Antibody Titer Assay Drug Tolerance)

Figure 7. (a) Incidence and mean titers of PEG IgG and (b) incidence and mean titers of PEG IgM with pegvaliase concentration over time in Study 165-301

(a) Incidence and mean titers of PEG IgG



(b) Incidence and mean titers of PEG IgM



(Source of data: Reviewer's analysis based on data from Tables 14.2.1.3, 14.3.6.3.2, and 14.3.6.3.3 of 165-301 CSR)

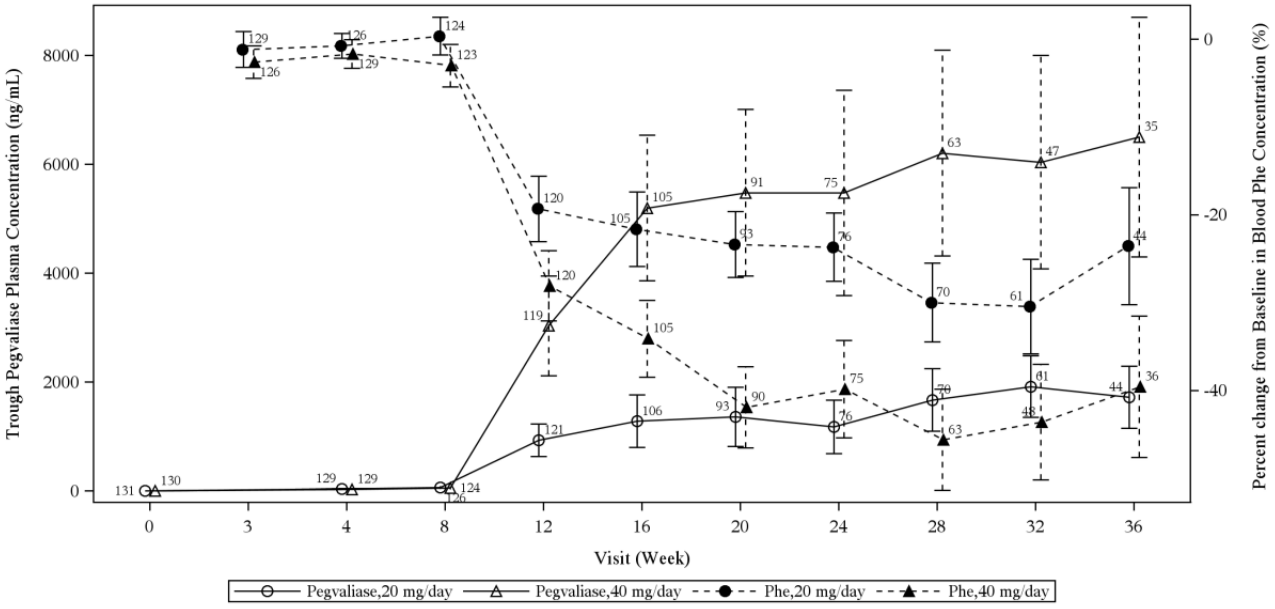
1.2 Clinical Pharmacokinetic (PK) and Pharmacodynamic (PD) Assessments

The PK and PD of pegvaliase were assessed in seven clinical studies. This section describes the PK/PD results in the Phase 3 trials in which the to-be-marketed PFS drug product was used. The VS drug product was also used in the Phase 3 studies. In 165-301, both the VS drug product and PFS drug product were used. In 165-302, VS was used in Parts 1, 2, and 3A, whereas the PFS drug product was used in Parts 3B and 4. Note that the Phase 3 PK assay used to analyze PK samples was different from the Phase 1 and Phase 2 PK assays.

1.2.1 Study 165-301

Plasma pegvaliase and blood Phe concentrations by randomized dose group in 165-301 are presented in **Table 5**. Mean plasma pegvaliase concentration was not detectable with minimal reduction in blood Phe concentration from baseline in the first 8 weeks (**Figure 8**). After Week 8, as subjects titrated to receiving higher doses, mean trough pegvaliase concentration increased with greater reduction in Phe concentrations. Mean trough pegvaliase concentration and Phe reduction appeared to reach steady state by Week 24.

Figure 8. Mean Trough Pegvaliase Concentration and Percent Change from Baseline in Phe level Over Time by Randomized Dose Group



(Source of data: Figure 2.7.2.2.6.1 Module 2.7.2 Summary of Clinical Pharmacology Studies or Figure 14.2.2.1 of 165-301 CSR)

Table 5. Trough pegvaliase concentration and blood Phe concentration over time by randomized dose group

	Trough Pegvaliase Concentration (ng/mL)			Blood Phe Concentration (µmol/L)		
	20 mg/day (N = 131)	40 mg/day (N = 130)	Total (N = 261)	20 mg/day (N = 131)	40 mg/day (N = 130)	Total (N = 261)
Baseline						
N	131	130	261	131	130	261
Mean (SD)	0.9 (10.8)	0 (0)	0.5 (7.68)	1241 (390)	1224 (384)	1233 (386)
Median	0	0	0	1253	1216	1221
Min, Max	0 , 124	0 , 0	0 , 124	285 , 2186	483 , 2330	285 , 2330
Week 4						
N	129	129	258	126	129	255
Mean (SD)	38.6 (125)	28.7 (105)	33.6 (116)	1224 (381)	1185 (372)	1204 (376)
Median	0	0	0	1228	1219	1223
Min, Max	0 , 736	0 , 662	0 , 736	201 , 2257	247 , 2137	201 , 2257
Week 8						
N	126	124	250	124	123	247
Mean (SD)	64.8 (270)	57.8 (290)	61.3 (280)	1228 (375)	1168 (428)	1198 (402)
Median	0	0	0	1206	1173	1200
Min, Max	0 , 2,200	0 , 2,930	0 , 2,930	242 , 2192	0 , 2230	0 , 2230
Week 12						
N	121	119	240	120	120	240
Mean (SD)	933 (3,349)	3,033 (10,085)	1,974 (7,547)	997 (514)	859 (534)	928 (528)
Median	0	0	0	1032	966	979
Min, Max	0 , 21,200	0 , 77,400	0 , 77,400	0 , 1951	0 , 2025	0 , 2025
Week 16						
N	106	105	211	105	105	210
Mean (SD)	1,279 (5,054)	5,199 (13,807)	3,230 (10,537)	953 (494)	799 (564)	876 (534)
Median	0	0	0	942	867	914
Min, Max	0 , 35,900	0 , 72,600	0 , 72,600	0 , 1997	0 , 1948	0 , 1997
Week 20						
N	93	91	184	93	90	183
Mean (SD)	1,360 (5,338)	5,479 (14,683)	3,397 (11,163)	933 (487)	678 (552)	808 (534)
Median	0	0	0	992	683	850
Min, Max	0 , 44,300	0 , 94,400	0 , 94,400	0 , 1795	0 , 1915	0 , 1915
Week 24						
N	76	75	151	76	75	151

Mean (SD)	1,177 (4,339)	5,473 (16,405)	3,311 (12,117)	929 (449)	668 (548)	800 (516)
Median	0	0	0	955	698	822
Min, Max	0 , 32,400	0, 108,000	0, 108,000	0 , 1881	0 , 1842	0 , 1881
Week 28						
N	70	63	133	70	63	133
Mean (SD)	1,673 (4,881)	6,205 (15,075)	3,820 (11,151)	853 (500)	598 (536)	732 (531)
Median	0	144	0	866	563	713
Min, Max	0 , 26,900	0 , 92,200	0 , 92,200	0 , 1818	0 , 1649	0 , 1818
Week 32						
N	61	47	108	61	48	109
Mean (SD)	1,918 (4,460)	6,041 (13,503)	3,712 (9,683)	801 (500)	586 (482)	706 (501)
Median	0	175	114	840	622	702
Min, Max	0 , 19,900	0 , 68,000	0 , 68,000	0 , 1732	0 , 1690	0 , 1732
Week 36						
N	44	35	79	44	36	80
Mean (SD)	1,722 (3,846)	6,502 (13,085)	3,840 (9,407)	868 (502)	624 (531)	759 (526)
Median	61.5	178	123	897	639	788
Min, Max	0 , 14,500	0 , 64,500	0 , 64,500	0 , 1738	0 , 1606	0 , 1738

(Source of data: Table 14.2.1.3 and 14.2.1.1.1.1 of 165-301 CSR)

1.2.2 Study 165-302 Part 1

At Part 1 Week 5, subjects in the 40 mg/day group had approximately 5-fold higher mean trough plasma pegvaliase concentration than the 20 mg/day group (**Table 6**). The inter-subject variability of the trough plasma pegvaliase concentration was high in both groups, however.

Table 6. Trough pegvaliase concentrations in Study 165-302 Part 1

Part 1 (Open-Label Blood Phe Assessment)			
		20 mg/day Active n=86	**40 mg/day Active n=78**
Part 1, Week 5	n	56	40
	Mean (SD)	944.7 (2364.96)	4747.2 (9451.28)
	Median	80.1	159.5
	Min, Max	0 , 12200	0 , 37000
Part 1, Week 9	n	37	26
	Mean (SD)	986.3 (2370.31)	2161.2 (5851.00)
	Median	0.0	431.0
	Min, Max	0 , 11200	0 , 28600

(Source of data: Table 11.1.1.2.1.1.1 of 165-302 CSR)

1.2.3 Study 165-302 Part 2

Table 7 presents pegvaliase trough concentrations in subjects who had ≥ 20% reduction in blood Phe concentrations from naïve baseline levels on a dose of 20 or 40 mg/day in 165-302 Part 2. Mean trough plasma pegvaliase concentrations were similar between subjects randomized to placebo and treatment active groups at baseline. Mean trough plasma pegvaliase concentration in the placebo groups decreased over time after switching to placebo in Part 2; all subjects had undetectable plasma pegvaliase concentration at Weeks 4 and 8 except for two subjects at Week 4 and one subject at Week 8. The plasma pegvaliase concentrations in subjects in the active groups were sustained during the 8-week duration of Part 2.

Refer to Sections 7 and 8 of the Multi-Disciplinary Review for the efficacy analysis results of blood Phe concentration in Part 2 of 165-302.

Table 7. Trough pegvaliase concentrations in Study 165-302 Part 2

Part 2 (Randomized, Double-Blind Discontinuation)					
		20 mg/day Active n=29	**40 mg/day Active n=29**	**20 mg/day Placebo n=14**	**40 mg/day Placebo n=14**
Part 2, Baseline	n	29	25	14	14
	Mean (SD)	5566.0 (8830.95)	7342.4 (12514.77)	4309.5 (6906.24)	6841.3 (14992.83)
	Median	1510.0	1410.0	823.0	966.5
	Min, Max	0 , 30600	0 , 56200	0 , 23500	0 , 43300
Part 2, Week 4	n	29	29	14	14
	Mean (SD)	6251.4 (10114.67)	9026.8 (15448.02)	26.1 (97.55)	1278.6 (4783.98)
	Median	1180.0	1290.0	0.0	0.0
	Min, Max	0 , 38400	0 , 57600	0 , 365	0 , 17900
Part 2, Week 8	n	26	23	13	10
	Mean (SD)	7431.3 (10350.86)	5708.5 (8919.25)	73.5 (264.87)	0.0 (0.00)
	Median	1705.0	1250.0	0.0	0.0
	Min, Max	0 , 35900	0 , 27800	0 , 955	0 , 0

(Source of data: Table 11.1.1.2.1.2.1 of 165-302 CSR)

1.2.4 Study 165-302 Part 3

Part 3 was a two-part open-label period designed to evaluate multiple-dose PK/PD in subjects who self-administer pegvaliase using the VS drug product and PFS drug product. Subjects received VS drug product for a week in Part 3A, and intensive PK and PD assessments were performed for 24 hours on Week 1 Day 7. Subjects entered Part 3B at Week 2 and transitioned to PFS drug product for 4 weeks. At Week 6, subjects temporarily halted dosing for 1 week and performed intensive PK/PD assessments.

Subjects who were randomized to the active group of 20 or 40 mg/day in Part 2 had increased mean and median trough pegvaliase concentrations at Week 4 and Week 5/6 after switching to PFS compared with that at Week 1 when subjects received VS. Subjects who were randomized to placebo in Part 2 had higher mean trough concentrations with the PFS in the 40-mg group. However, the increase in mean trough exposure at the 40-mg dose level was also associated with increased variability in exposure **(Table 8**).

Table 8. Trough pegvaliase concentrations in Study 165-302 Part 3

	Drug Presentation		20 mg/day Active n=23	40 mg/day Active n=18	20 mg/day Switch from Placebo to Active n=11	40 mg/day Switch from Placebo to Active n=8
Part 3, Week 1	VS	n	17	15	7	8
		Mean (SD)	3984.0 (6335.32)	6442.0 (11500.93)	3733.3 (2740.06)	5064.6 (5168.70)
		Median	1960.0	2740.0	4070.0	4380.0
		Min, Max	0 , 24900	0 , 44100	123 , 7150	0 , 13800
Part 3, Week 4	VS	n	23	17	10	8
		Mean (SD)	6298.7 (10226.12)	8128.4 (13336.67)	2926.0 (2588.46)	15130.9 (25174.88)
		Median	2810.0	2520.0	2505.0	900.0
		Min, Max	0 , 45900	0 , 43000	0 , 7250	0 , 57900
Part 3, Week 5/6	PFS	n	20	15	9	8
		Mean (SD)	6810.7 (7148.64)	7199.6 (10008.21)	3297.2 (3795.15)	16776.7 (32278.11)
		Median	4055.0	3590.0	1540.0	791.0
		Min, Max	0 , 23100	0 , 32800	0 , 10700	88 , 91500

(Source of data: Table 11.1.1.2.1.3.1 of 165-302 CSR)

Serial PK/PD sampling was conducted in 57 subjects, 38 subjects in the active group and 19 in the placebo group. The mean steady-state plasma concentration-time profile was relatively flat with a low peak to trough ratio for VS presentation at Part 3A Week 1, whereas mean pegvaliase concentration peaked around four hours after dosing for the PFS at Part 3B Week 5 (**Figure 9**). Following one-week withdrawal from dosing in Week 6 of Part 3B, plasma pegvaliase concentration decreased and the percent in blood Phe reduction also decreased (**Figure 9**).

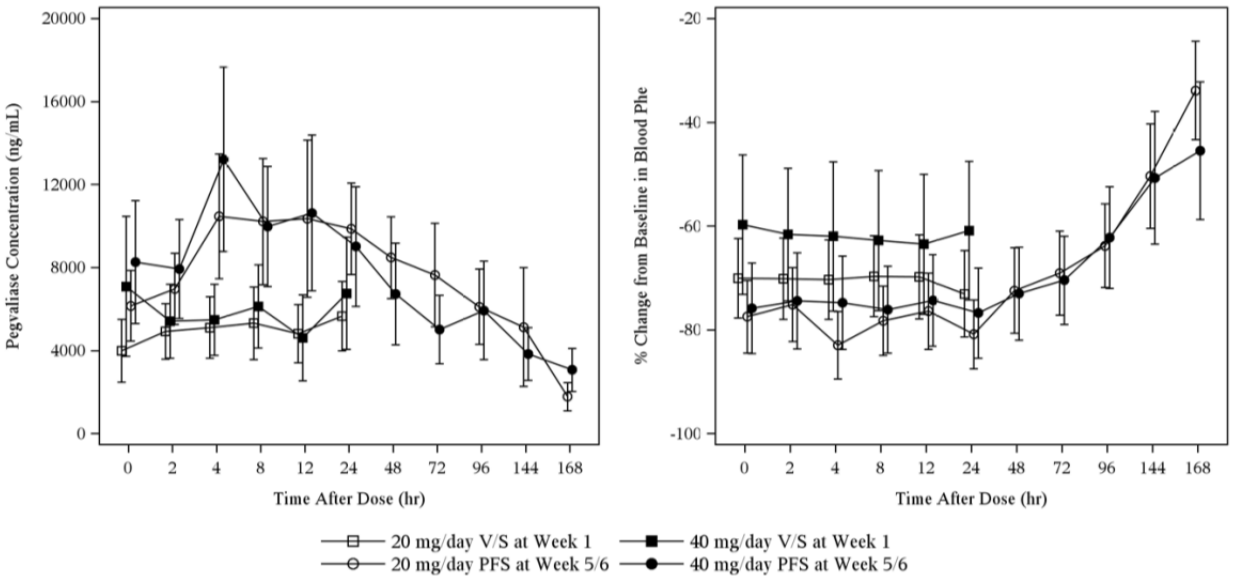
Table 9 summarizes the estimated steady-state PK parameters of pegvaliase for the VS and PFS presentations in all subjects.

Within subject comparison of pegvaliase exposure in 25 subjects (16 subjects in the 20 mg/day group and 9 subjects in the 40 mg/day group) showed that $AUC_{0\text{-}24h,ss}$ and $C_{max,ss}$ was 52% and 75% higher, respectively, for the PFS compared to the VS drug presentation. Mean blood Phe reduction Phe reduction was 81.2 % with the VS presentation and 83.0% with the PFS presentation in these subjects.

Figure 9. Plot of PK/PD profile at Part 3 Week 1 and Part 3 Week 5/6 for subjects in the active treatment groups of Study 165-302

PK: Pegvaliase concentrations

PD: % Blood Phe reduction from baseline



(Source of data: Figure 14.2.7.9 of 165-302 CSR)

Table 9. Steady state pegvaliase PK parameters by dose group in Part 3 Week 1 and Week 5/6 of Study 165-302

Parameter	Statistic	Vial and Syringe (Week 1)		Prefilled Syringe (Week 5/6)			
		Stayed on Active		Stayed on Active		Switched from Placebo to Active	
		20 mg/day n=19	40 mg/day n=14	20 mg/day n=20	40 mg/day n=18	20 mg/day n=11	40 mg/day n=8
$AUC_{0\text{-}24hr,ss}$ (ng*hr/mL)	n	12	7	17	12	7	7
	Mean	143039.7	206572.3	262181.5	246782.5	116564.8	454920.8
	SD	174109.69	212342.03	280378.19	338587.54	111571.12	649658.74
	Median	75500.3	109050.2	210350.9	109603.2	89065.4	30964.7
	Min, Max	16058 , 620176	18761 , 520775	5470 , 1126030	4507 , 1169209	10583 , 319763	5482 , 1425093
$C_{max,ss}$ (ng/mL)	n	16	10	17	15	8	8
	Mean	7098.4	10653.4	14042.6	16687.3	5334.5	23053.8
	SD	7971.62	13438.66	16254.69	19457.12	5209.85	35974.26
	Median	4535.0	6930.0	11400.0	8090.0	3435.0	1118.0
	Min, Max	824 , 32300	954 , 44100	255 , 68500	239 , 63800	536 , 15500	91 , 91500
$C_{trough,ss}$ (ng/mL) [a]	n	14	8	15	14	8	8
	Mean	6464.8	9727.3	11177.3	10446.0	4995.9	17408.5
	SD	6841.81	10438.97	8974.65	12723.89	5216.14	29014.29
	Median	4420.0	5035.0	11400.0	5265.0	2845.0	833.5
	Min, Max	647 , 25900	688 , 28500	210 , 29600	176 , 43100	347 , 15500	0 , 66100
$C_{avg,ss}$ (ng/mL)	n	12	7	17	12	7	7
	Mean	5960.0	8607.2	10924.2	10282.6	4856.9	18955.0
	SD	7254.57	8847.58	11682.42	14107.81	4648.80	27069.11
	Median	3145.8	4543.8	8764.6	4566.8	3711.1	1290.2
	Min, Max	669 , 25841	782 , 21699	228 , 46918	188 , 48717	441 , 13323	228 , 59379
$T_{max,ss}$ (hours)	n	16	10	17	15	8	8
	Mean	9.8	10.9	9.8	7.5	16.6	8.4
	SD	8.07	9.64	8.06	4.64	7.39	7.83
	Median	8.2	7.9	8.0	8.2	16.5	8.0

Parameter	Statistic	Vial and Syringe (Week 1)		Prefilled Syringe (Week 5/6)			
		Stayed on Active		Stayed on Active		Switched from Placebo to Active	
		20 mg/day n=19	40 mg/day n=14	20 mg/day n=20	40 mg/day n=18	20 mg/day n=11	40 mg/day n=8
	Min, Max	0 , 24	0 , 24	0 , 24	0 , 12	8 , 24	0 , 24
$t_{1/2}$[b] (hours)	n	NA	NA	13	8	5	3
	Mean	NA	NA	47.3	60.2	49.6	26.5
	SD	NA	NA	41.56	44.59	37.94	11.85
	Median	NA	NA	26.9	56.1	39.1	28.6
	Min, Max	NA	NA	14 , 132	14 , 127	15 , 115	14 , 37
CL_{ss}/F (mL/hr)	n	12	7	17	12	7	7
	Mean	384.1	657.6	394.4	1246.3	487.9	2509.0
	SD	365.11	763.33	866.36	2457.93	638.88	3012.89
	Median	267.2	366.8	95.1	406.2	224.6	1291.8
	Min, Max	32 , 1246	77 , 2132	18 , 3657	34 , 8876	63 , 1890	28 , 7296
Vdz/F[b] (mL)	n	NA	NA	13	5	4	3
	Mean	NA	NA	26448.3	22240.3	34518.3	72949.5
	SD	NA	NA	64806.23	19706.48	43550.85	70141.70
	Median	NA	NA	5997.8	20627.0	14259.4	69298.7
	Min, Max	NA	NA	1798 , 240924	3126 , 49487	9797 , 99757	4705 , 144845

(Source of data: Table 11.1.1.2.2.1 of 165-302 CSR)

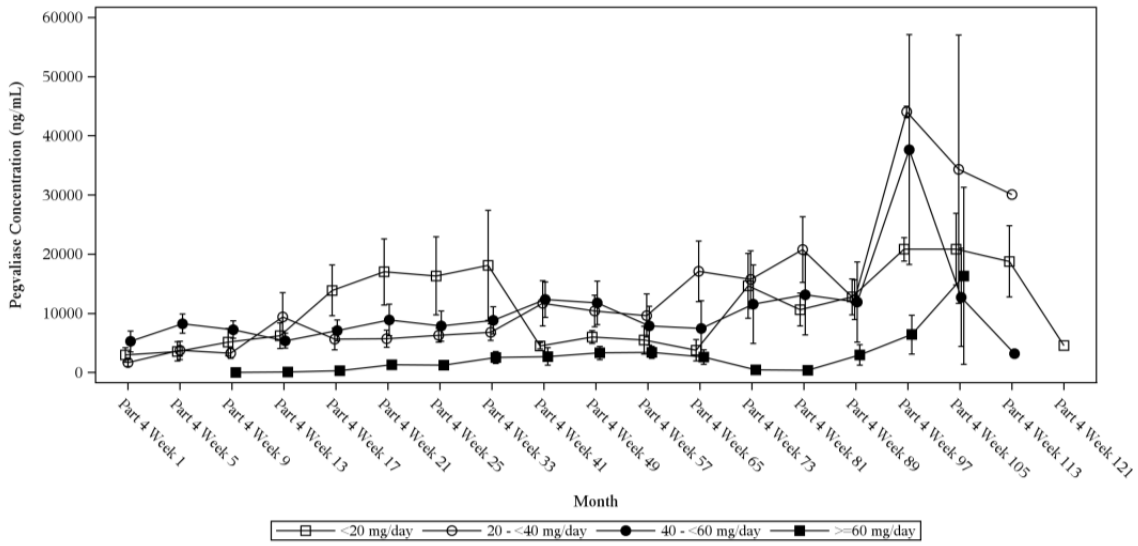
1.2.5 Study 165-302 Part 4

Because doses were adjustable in Part 4, the Applicant pooled the mean concentrations into dose categories (< 20 mg, 20 to < 40 mg, 40 to < 60 mg, and ≥ 60 mg) as presented in **Figure 10**.

Reviewer's Comments

Because subjects from different studies and different parts of 165-302 were enrolled in Part 4, the interpretation of the concentration data is challenging and the comparison of pegvaliase concentrations between treatment groups should be interpreted with caution.

Figure 10. Mean (SE) plot of trough pegvaliase concentration over time in Study 165-302 Part 4



(Source of data: Figure 14.2.7.8 of 165-302 CSR)

1.3 Dose-Response and Exposure-Response Analyses

Overall, dose-response (D-R) relationship in 165-301 suggests a trend of higher blood Phe reduction in the 40 mg/day group compared to the 20 mg/day. This relationship supports the escalation of daily dose to 40 mg if a minimum of 20% blood Phe reduction is not achieved in patients who were treated with 20 mg/day.

Study 165-301 was designed to evaluate a dose-response relationship in adult subjects with PKU dosed at two fixed maintenance doses (i.e., 20 mg/day and 40 mg/day). There were no meaningful differences in demographics or other baseline characteristics between the two randomized dose groups in the intention-to-treat (ITT) population (**Table 10** and **Table 11**). The median and 1st quartile percent reduction in blood Phe concentration were higher in the 40 mg/day group compared to the 20 mg/day group in the titration and maintenance phases (**Figure 11**).

Figure 12 depicts the percent blood Phe reduction by randomized dose including data only when subjects reached their randomized target dose. A similar trend of higher median blood Phe reduction in the 40 mg/day group compared to 20 mg/day group was observed. The effect of last observation carried forward (LOCF) imputation on missing data did not affect this trend either.

Table 10. Demographic characteristics for each randomized dose group in 165-301 ITT Population

Demographic Characteristic	20 mg/day (n = 131)	40 mg/day (n = 130)	Total (n = 261)
Age at enrollment (years)			
Mean (SD)	30.24 (8.63)	28.05 (8.77)	29.15 (8.75)
Median	29.00	26.00	28.00
Min, Max	16.00, 52.00	16.00, 55.00	16.00, 55.00
16 - < 18	5 (3.8%)	6 (4.6%)	11 (4.2%)
18 - < 66	126 (96.2%)	124 (95.4%)	250 (95.8%)
Sex (n [%])			
Female	62 (47.3%)	68 (52.3%)	130 (49.8%)
Male	69 (52.7%)	62 (47.7%)	131 (50.2%)
Race			
American Indian or Alaska Native (n [%])	0	1 (0.8%)	1 (0.4%)
Black or African American (n [%])	1 (0.8%)	2 (1.5%)	3 (1.1%)
Native Hawaiian or Pacific Islander (n [%])	0	0	0
White (n [%])	130 (99.2%)	124 (95.4%)	254 (97.3%)
Other (n [%])	0	2 (1.5%)	2 (0.8%)
Missing (n [%])	0	1 (0.8%)	1 (0.4%)
Ethnicity			
Hispanic or Latino (n [%])	6 (4.6%)	1 (0.8%)	7 (2.7%)
Not Hispanic or Latino (n [%])	125 (95.4%)	128 (98.5%)	253 (96.9%)
Missing (n [%])	0	1 (0.8%)	1 (0.4%)
Weight, kg			
n	131	129	260
Mean (SD)	82.0 (20.49)	78.9 (20.85)	80.5 (20.68)
Median	81.1	74.9	77.2
Min, Max	45.3, 139.2	41.5, 135.9	41.5, 139.2
Height, cm			
n	131	129	260
Mean (SD)	168.1 (9.25)	168.1 (9.68)	168.1 (9.45)
Median	167.6	167.6	167.6
Min, Max	143.5, 192.0	149.0, 190.0	143.5, 192.0
BMI, kg/m^2			
n	131	129	260
Mean (SD)	29.0 (6.96)	27.8 (6.48)	28.4 (6.74)
Median	28.5	26.9	27.7
Min, Max	17.1, 47.3	17.2, 46.8	17.1, 47.3

BMI, body mass index; Max, maximum; Min, minimum; SD, standard deviation.
(Source of data: Table 10.2.1 of 165-301 CSR)

Table 11. Baseline characteristics for each randomized dose group in 165-301 ITT Population

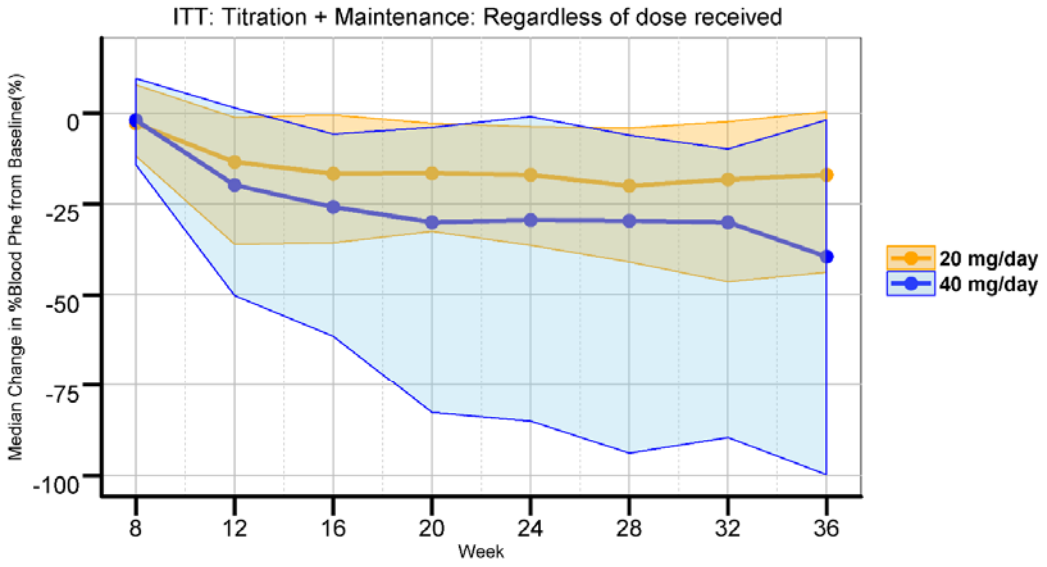
Baseline Characteristic	20 mg/day (n = 131)	40 mg/day (n = 130)	Total (n = 261)
Baseline blood Phe, µmol/L			
n	131	130	261
Mean (SD)	1241.0 (389.70)	1224.4 (384.28)	1232.7 (386.36)
Median	1253.0	1215.5	1221.0
Number of subjects on restricted diet (n [%])[a]	22 (16.8%)	19 (14.6%)	41 (15.7%)
Subjects taking protein from medical food	75 (57.3%)	74 (56.9%)	149 (57.1%)
Baseline protein from intact food (g)			
n	127	123	250
Mean (SD)	39.1 (27.27)	37.9 (28.30)	38.5 (27.73)
Median	29.8	30.3	29.9
Baseline neurocognitive/neuropsychiatric scores			
ADHD-RS total score (n)	85	84	169
Mean (SD)	15.6 (10.92)	15.9 (9.31)	15.8 (10.13)
ADHD-RS inattention (n)	129	124	253
Mean (SD)	10.0 (6.59)	9.5 (5.60)	9.8 (6.12)
ADHD-RS inattention (score > 9) (n)	59	57	116
Mean (SD)	16.0 (4.48)	14.6 (3.49)	15.3 (4.06)
POMS total mood disturbance score (n)	86	84	170
Mean (SD)	36.8 (30.91)	34.6 (30.54)	35.7 (30.66)
PKU POMS total score (n)	86	84	170
Mean (SD)	16.6 (13.46)	15.1 (13.01)	15.9 (13.21)
PKU POMS confusion (n)	86	84	170
Mean (SD)	3.8 (2.65)	4.1 (2.70)	4.0 (2.67)

ADHD-RS, Attention Deficit Hyperactivity Disorder Rating Scale; BMI, body mass index; Max, maximum; Min, minimum; PKU, phenylketonuria; POMS, Profile of Mood States; SD, standard deviation.

[a] Subjects were considered to be on a restricted diet if greater than 75% of their total protein intake was from medical food. Total protein intake was defined as the sum of the protein intake from medical food and protein intake from intact food.

(Source of data: Table 10.2.2 of 165-301 CSR)

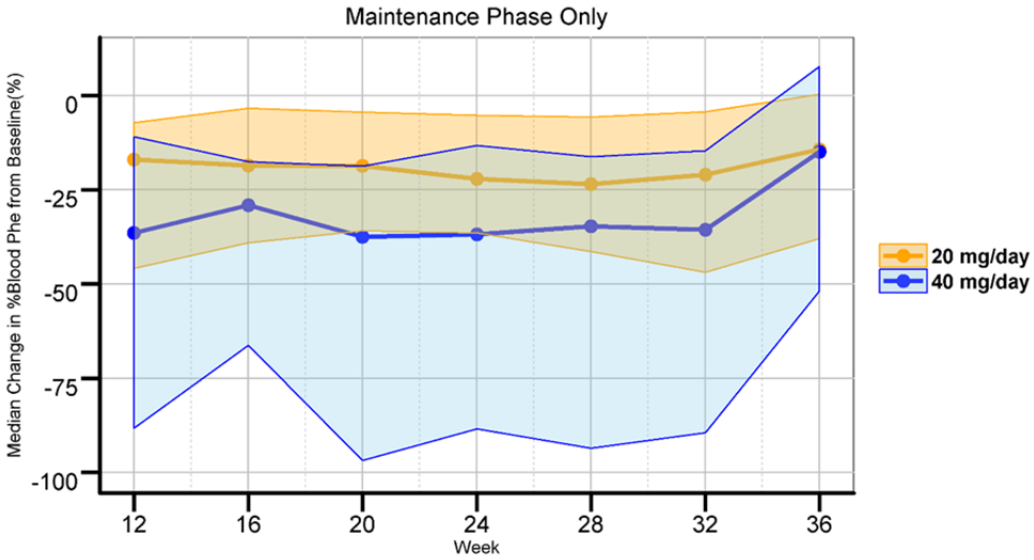
Figure 11: % Change in blood Phe reduction from baseline by dose group in the ITT Population during the titration and maintenance phases of Study 165-301



Treatment	Week 8	Week 12	Week 16	Week 20	Week 24	Week 28	Week 32	Week 36
20 mg/day	117	118	119	118	115	115	112	42
40 mg/day	122	123	121	114	111	109	106	35

Solid colored line: Median. Shaded Area: 1st and 3rd quartile. Table: Number of subjects by dose group at each assessment timepoint.
(Source of data: Reviewer's analysis based on datasets "adeff.xpt" and "adex.xpt" from 165-301)

Figure 12. % Change in blood phe reduction from baseline by dose group in patients who reached the planned maintenance dose in Study 165-301



Treatment	Week 12	Week 16	Week 20	Week 24	Week 28	Week 32	Week 36
20 mg/day	89	102	100	102	102	99	38
40 mg/day	67	78	83	81	80	78	28

Solid colored line: Median. Shaded Area: 1st and 3rd quartile. Table: Number of subjects by dose group at each assessment timepoint. (Source of data: Reviewer's analysis based on datasets "adeff.xpt" and "adex.xpt" from 165-301)

A mixed-effect repeated measures (MMRM) model was used to further explore the difference of treatment effect between the two maintenance doses. Results showed a trend supportive of higher blood Phe reduction in the 40 mg/day group after adjusting for significant predictors including blood Phe concentration at baseline and protein intake from intact food at baseline. The least squares mean difference between the 20 mg/day group and 40 mg/day group was 9.72% with a 95% confidence interval (CI) of 0.75% to 18.69% (**Table 12**). A separate MMRM analysis based on the population of subjects who entered the maintenance period only showed a similar favorable treatment effect of the 40 mg/day group. The least squares mean difference between the two dose groups was 17.77% (95% CI: 7.23%, 28.3%).

The time to three consecutive blood Phe reduction to 360 µmol/L, 600 µmol/L, or 20% reduction from baseline was compared between the 20 mg/day and 40 mg/day groups. A trend of earlier blood Phe reduction was identified in patients treated with 40 mg/day compared to 20 mg/day group (**Figure 13**). The hazard ratio (HR) of the time to three consecutive blood Phe reduction to 360 umol/L and 20% blood Phe reduction was 1.97 (95% CI: 1.09, 3.56) and 1.36 (95% CI: 0.96, 1.92), respectively, comparing 40 mg/day to 20 mg/day based on Cox Proportional analysis after adjusting for baseline predictors (**Table 13**). The Kaplan-Meier (K-M)

curves and parameter estimates were very similar after excluding values imputed using LOCF method (**Table 14** and **Figure 14**).

Table 12: Mixed model repeated measures of percent change from baseline blood Phe concentration

	LS Mean (95% CI)	Difference in LS Mean (95% CI)
MMRM: ITT Population in titration and maintenance phase		
20 mg/day	-12.37 (-20.49, -4.24)	9.72 (0.75, 18.69)
40 mg/day	-22.08 (-30.27, -13.9)	.
MMRM: Maintenance Phase only		
20 mg/day	-16.81 (-25.21, -8.4)	17.77 (7.23, 28.3)
40 mg/day	-34.57 (-43.38, -25.76)	.
MMRM: Maintenance Phase only no LOCF		
20 mg/day	-18.17 (-26.84, -9.5)	16.86 (6.43, 27.29)
40 mg/day	-35.03 (-44.08, -25.99)	.

(Source of data: Reviewer's analysis based on datasets "adeff.xpt" and "adex.xpt" from 165-301)

Table 13: Parameter estimates of final dose-response model comparing 20 mg/day versus 40 mg/day in time to blood Phe reduction

Covariate	HR	Lower 95% CI	Upper 95% CI	P-value
Time to 3 consecutive blood Phe concentration <= 360 umol/L				
Protein intake from intact food at BL (m)	0.983	0.97	0.997	0.0176
40 mg/day vs 20 mg/day	1.97	1.09	3.56	0.0252
Time to 3 consecutive blood Phe concentration <= 600 umol/L				
Phe Blood at BL (nmol/L)	0.313	0.149	0.658	0.00215
Protein intake from intact food at BL (m)	0.984	0.971	0.998	0.0218
40 mg/day vs 20 mg/day	1.47	0.93	2.32	0.0994
Time to 3 consecutive 20% blood Phe reduction from baseline				
Protein intake from intact food at BL (m)	0.995	0.988	1	0.136
40 mg/day vs 20 mg/day	1.36	0.962	1.92	0.0815

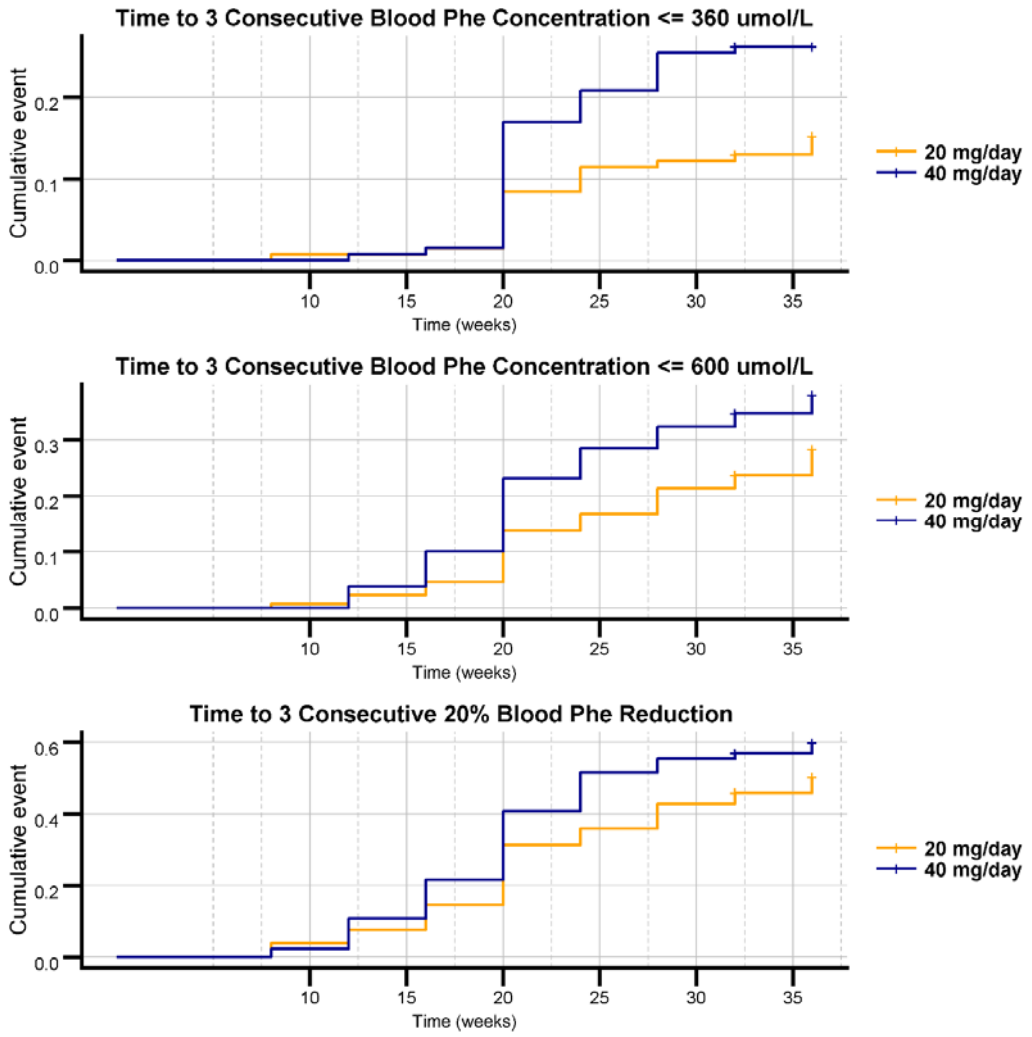
(Source of data: Reviewer's analysis based on datasets "adeff.xpt" and "adex.xpt" from 165-301)

Table 14: Parameter estimates of final dose-response model comparing 20 mg/day versus 40 mg/day in time to blood Phe reduction (excluding LOCF)

Covariate	HR	Lower 95% CI	Upper 95% CI	P-value
Time to 3 consecutive blood Phe concentration <= 360 umol/L				
40 mg/day vs 20 mg/day	2.22	1.18	4.16	0.013
Time to 3 consecutive blood Phe concentration <= 600 umol/L				
Total Dietary Phe Intake at Baseline (g)	0.726	0.524	1.01	0.0553
Phe Blood at BL (nmol/L)	0.273	0.118	0.635	0.00255
40 mg/day vs 20 mg/day	1.51	0.922	2.47	0.102
Time to 3 consecutive 20% blood Phe reduction from baseline				
40 mg/day vs 20 mg/day	1.37	0.94	1.98	0.0987

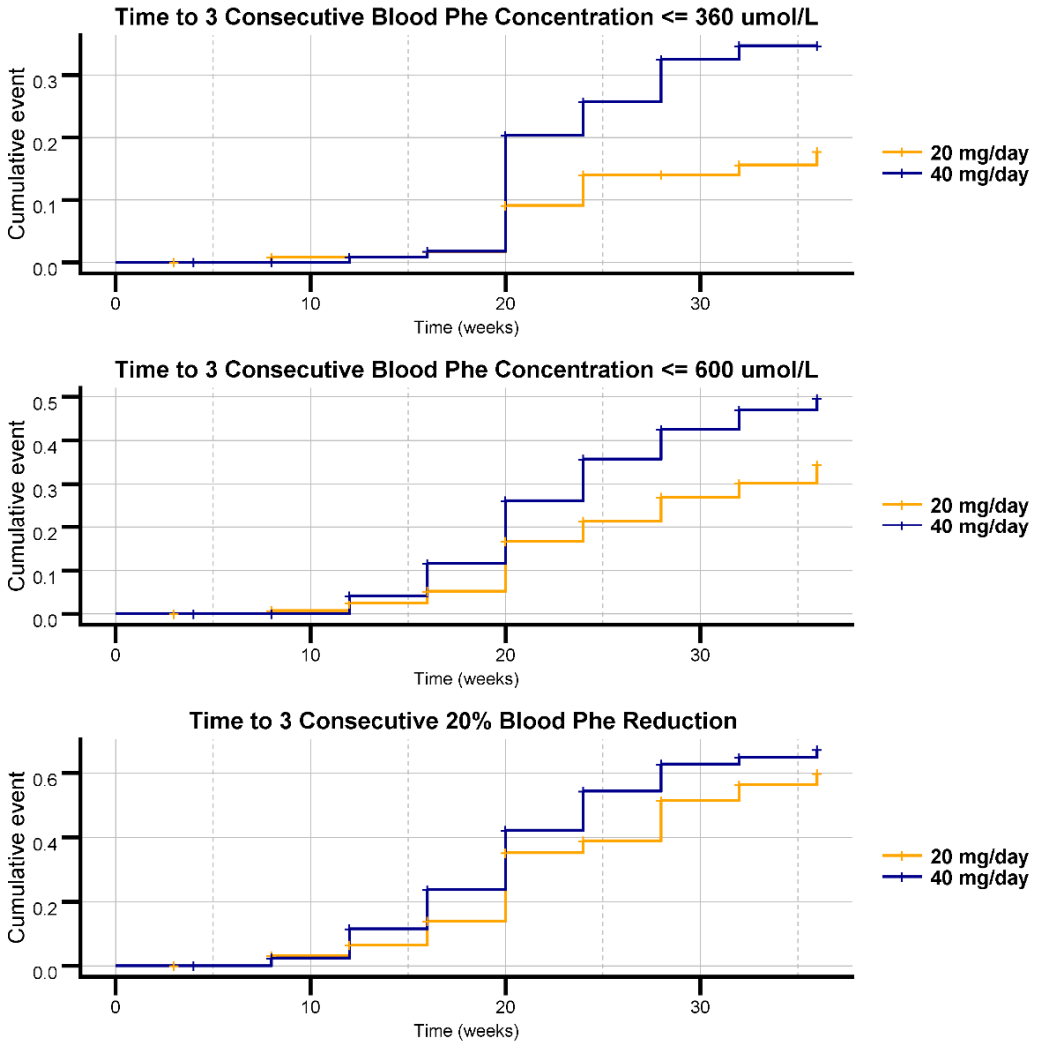
(Source of data: Reviewer's analysis based on datasets "adeff.xpt" and "adsl.xpt" from 165-301)

Figure 13: K-M curve of time to blood Phe reduction by dose group in the ITT Population in Study 301



(Source of data: Reviewer's analysis based on datasets "adeff.xpt" and "adsl.xpt" from 165-301)

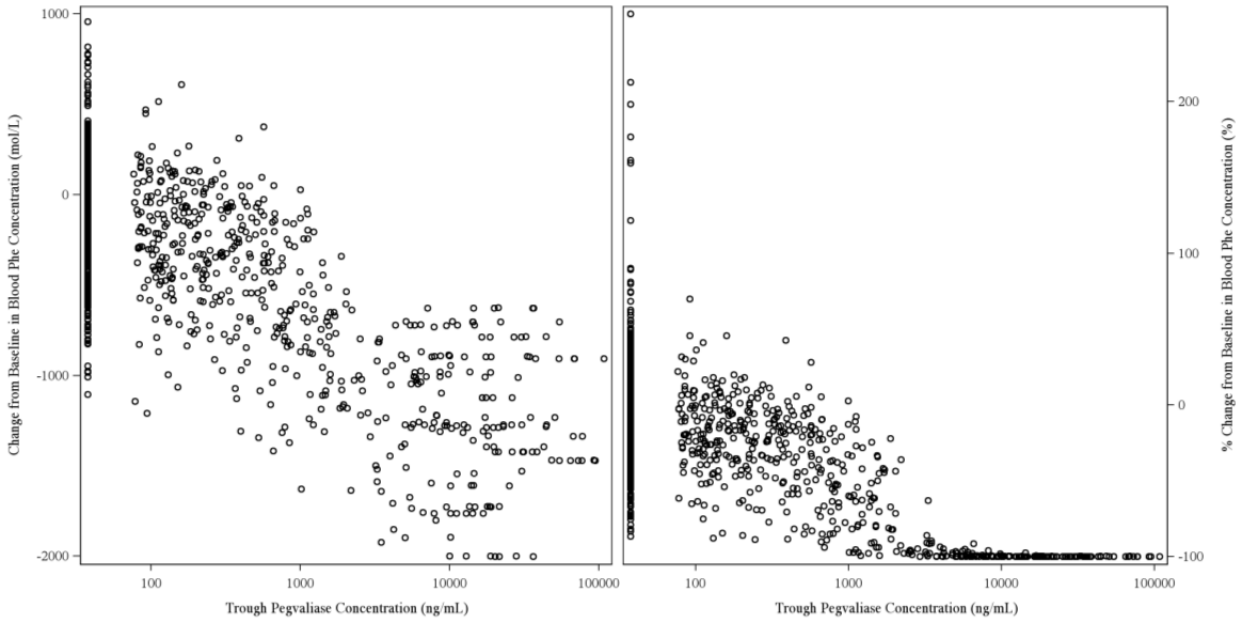
Figure 14: K-M curve of time to blood Phe reduction by dose group in the ITT Population in Study 301 (excluding LOCF)



(Source of data: Reviewer's analysis based on datasets "adeff.xpt" and "adsl.xpt" from 165-301)

The exposure-response (E-R) relationship proposed by the applicant suggested the absolute change and percent (%) change from baseline in blood Phe concentration inversely correlated with pegvaliase trough concentration (**Figure 15** and **Figure 16**). Blood Phe reduction increased with increasing pegvaliase trough concentration, with blood Phe reduction reaching a plateau at pegvaliase trough concentration of around 2500 ng/mL (as reported in the population PK analysis report).

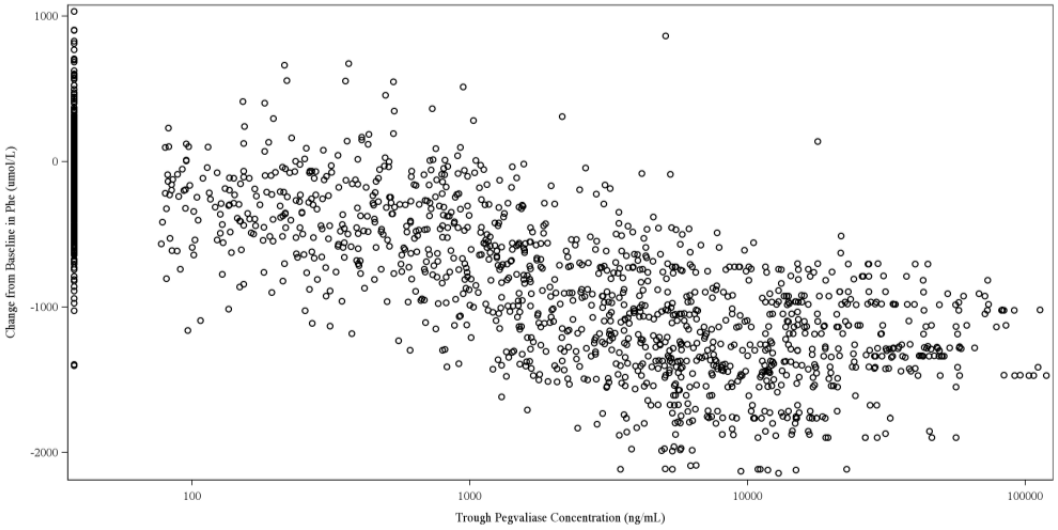
Figure 15: Scatter plot of change (% change) from baseline in blood Phe concentration and observed pegvaliase trough concentration in Study 165-301



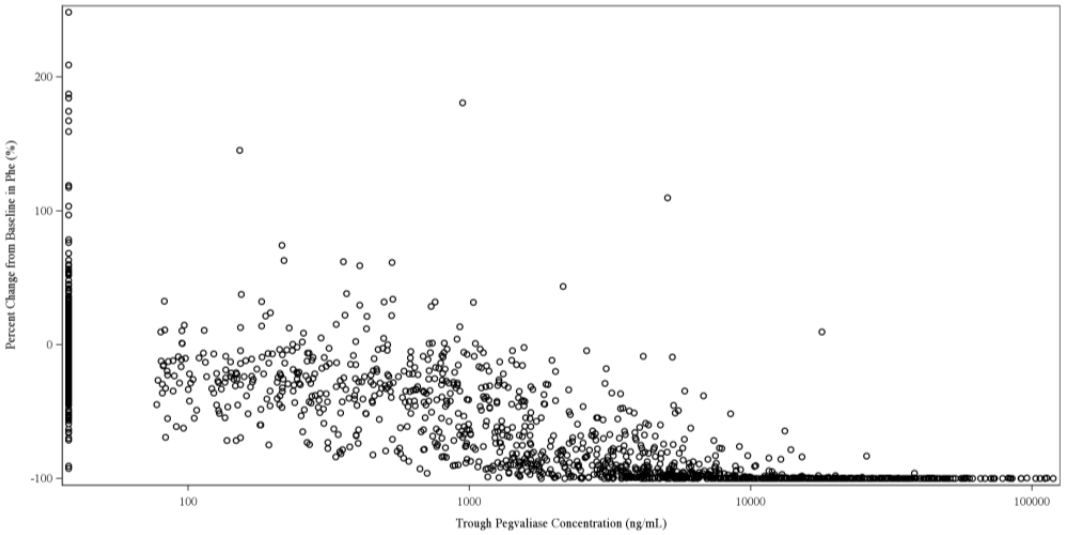
(Source of data: Figure 14.2.2.3 of 165-301)

Figure 16: Scatter plot of change (% change) from baseline in blood Phe concentration and observed pegvaliase trough concentration in Study 165-302

Change from baseline in Phe



% Change from baseline in Phe



(Source of data: Figure 14.2.7.16 of 165-302 CSR)

1.4 Immunogenicity

1.4.1 Immunogenicity Assessment Strategy

This section focuses on the immunogenicity assessment of the Phase 3 studies (i.e., 165-301 and 165-302). Immunogenicity results from Phase 1 or Phase 2 studies are included as necessary.

The Phase 3 Population included 273 subjects who participated in 165-301 and then 165-302 (n = 261), as well as 12 subjects who entered 165-302 from the Phase 2 studies 165-205 (n = 4) and PAL-003 (n = 8). Due to the use of different immunogenicity assays in Phase 2, data for the initial treatment period from these 12 subjects are not included in this review. Hence, immunogenicity data from the 36-week study 165-301 are used to represent the data of the Phase 3 Population during I/T/M periods. Data for the 12 subjects during later treatment period after enrollment in 165-302 are included in this evaluation.

Sampling for immunogenicity assessment was performed routinely at specified time points throughout the Phase 3 studies (**Table 15**).

In all Phase 3 subjects, the Applicant also evaluated complement activating CICs (i.e., IgG CIC and IgM CIC) using separate C3d ELISA assays. Approximately six timepoints per subject were tested and results are presented by windowing data into treatment week intervals from time of first dose (Treatment Week Intervals: Baseline, Week 12, Week 36, Week 60, Week 84, Week 108, Week 132, Week 156, Week 180, etc.) Data at different time points reflect a smaller and variable sample size due to the limited number of subjects with data after Week 84. Phase 2 subjects entering into 165-302 did not have a baseline CIC value due to the age of the sample, or due to lack of consent for exploratory research.

Table 15. Immunogenicity assessment schedule

	Anti-PAL antibodies, anti-PEG antibodies, TAb, NAb, anti-pegvaliase and anti-PAL IgE	C3, C4
165-301	Baseline, Weeks 3, 4, 8, 12, 16, 20, 24, 32, 36	Baseline, Weeks 4, 8, 12, 16, 20, 24, 28, 32
165-302		
Part 1	Baseline (Week 1), Weeks 5, 9	Baseline (Week 1) Weeks 5, 9
Part 2	Baseline (Week 1), Weeks 4, 8	Baseline (Week 1), Weeks 4, 8
Part 3	Week 4	Week 4
Part 4	Weeks 1, 5, 9, 13, 17, 21, 25, every 8 weeks thereafter	Weeks 1, 9, 17, 25, every 8 weeks thereafter

(Source of data: adopted from Table 2.1.2.1 of IRR, Table 8.5.1.1 of 165-301 CSR, and Table 7.5.1.1, 7.5.1.2, 7.5.1.3, and 7.5.1.4 of 165-302 CSR)

Upon request from the Agency dated November 22, 2016, the Applicant evaluated anti-pegvaliase IgG4 in all Phase 3 subjects. Up to 11 longitudinal time points were tested per subject, with the number of time points tested dependent on each subject's time on treatment at the time of the 120-Day data cut on May 6, 2017. Anti-pegvaliase IgG4 antibody results are presented by windowing data into treatment week intervals from time of first dose for all subjects (Treatment Week Intervals: Baseline, Week 4, Week 12, Week 24, Week 48, Week 72, Week 96, Week 120, Week 144, etc.). Data at different time points reflect a smaller and variable sample size due to the limited number of subjects with data after Week 120.

Reviewer's Comments

Section 6.2 of the proposed labeling describes immunogenicity data (including incidences and time profiles) for the I/T/M Population (i.e., 24 subjects enrolled from Study 165-205 and 261 subjects from Study 165-301); it includes data generated from both the Phase 2 and Phase 3 ADA assays. Hence, the immunogenicity data in the labeling are slightly different from the immunogenicity data obtained from the Phase 3 Population as described in this review. Nonetheless, the overall immunogenicity data are very similar between the I/T/M Population and the Phase 3 Population and did not change the conclusions of the review.

1.4.2 Pre-existing Antibodies (anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, TAb, NAb)

The incidences and mean antibody titers over time for anti-PAL IgG and IgM, anti-PEG IgG and IgM, TAb, and NAb in 165-301 are summarized in **Table 16** to **Table 19**. At pre-treatment baseline, the incidence of anti-PAL IgM was 50.2% with mean (median) titers of 244 (131). The incidences of anti-PEG IgG and PEG IgM were 52.5% and 44.8%, respectively, and mean (median) titers were 36 (10) and 178 (0), respectively.

PAL is a bacterial protein. The pre-existing anti-PAL IgM antibodies could be due to previous exposure to PAL protein via bacteria expressing PAL or other cross-reactive antigens. Similarly,

pre-existing anti-PEG IgG and anti-PEG IgM antibodies could be due to prior exposure to PEG and/or PEG derivatives.

1.4.3 Development of Anti-Drug Antibodies (anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, TAb, NAb) in 165-301

In Study 165-301, all subjects developed sustained TAb and anti-PAL IgG responses following treatment with pegvaliase; > 95% of subjects were tested positive for TAb and anti-PAL IgG antibodies starting from Week 8 (**Table 16** and **Figure 17**). Majority of the subjects developed anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, and NAb antibodies (**Table 16** and **Figure 17**).

Mean TAb titer peaked at Week 3 (mean = 67,137 [range: 0 to 1,060,000]; n = 255), then decreased slightly but was sustained through Week 36 (mean = 20,169 [range: 50 to 107,000]; n = 83) (**Table 19** and **Figure 18**). Anti-PAL IgG incidence and titer (mean = 911,507 [range: 150 to 8,857,350]; n = 197) generally peaked around 16 weeks (**Table 17** and **Figure 18**) and were sustained until the end of the study.

The incidence of anti-PAL IgM generally peaked at Week 8 – 12 (**Table 16**). Anti-PAL IgM titer (mean = 3,315 [range: 115 to 129,900]; n = 224) peaked at about Week 12, and then declined and reached steady state by Week 20 (mean = 955 [range:106 to 31,590]; n=165) (**Table 17** and **Figure 18**).

The incidence of NAb generally peaked at Week 20 – 24 (**Table 16**). NAb titer (mean = 540, range: 0 to 39,366; n = 195) peaked at Week 16 and remained stable through Week 36 (mean = 509 [range: 0 to 4,374]; n = 83) (**Table 19** and **Figure 18**). The mean titer-time profile was similar between anti-PAL IgG and NAb (**Figure 18**), suggesting that NAb may be a component of the anti-PAL IgG response.

The incidence of anti-PEG IgG peaked at Week 4 – 8 and then declined to below the incidence at baseline by Week 36 (**Table 16**). Mean anti-PEG IgG titer peaked at Week 3 (mean = 7,942 [range: 0 to 196,830]; n = 255) and then decreased to a mean titer value of 43 (range: 0 to 2,430; n = 83) by Week 36 (**Table 18** and **Figure 18**).

The incidence of anti-PEG IgM peaked at Week 8 – 12 then returned to slightly above baseline incidence by Week 36 (**Table 16**). Mean anti-PEG IgM titer peaked at Week 12 (mean = 47,618 [range: 0 to 5,360,000]; n = 224) and then decreased by Week 36 to a mean titer of 369 (range: 0 to 9,900; n = 36) (**Table 18** and **Figure 18**).

Table 16. Incidences of antibody positivity for the Phase 3 Population during the first 36 weeks after pegvaliase administration

Study Week	Total (N = 261)							
	Anti-PAL IgG	Anti-PAL IgM	Anti-PEG IgG	Anti-PEG IgM	TAb	NAb		
						Total	20 mg/day (N = 131)	40 mg/day (N = 130)
Baseline	17/259 (6.6%)	130/259 (50.2%)	136/259 (52.5%)	116/259 (44.8%)	80/259 (30.9%)	1/257 (0.4%)	0/128 (0.0%)	1/129 (0.8%)
W3	58/255 (22.7%)	195/255 (76.5%)	215/255 (84.3%)	218/255 (85.5%)	217/255 (85.1%)	0/255 (0.0%)	0/129 (0.0%)	0/126 (0.0%)
W4	90/257 (35.0%)	191/257 (74.3%)	238/256 (93.0%)	222/257 (86.4%)	236/257 (91.8%)	2/257 (0.8%)	1/128 (0.8%)	1/129 (0.8%)
W8	244/251 (97.2%)	247/252 (98.0%)	242/251 (96.4%)	242/252 (96.0%)	243/252 (96.4%)	35/248 (14.1%)	13/125 (10.4%)	22/123 (17.9%)
W12	224/225 (99.6%)	220/224 (98.2%)	147/225 (65.3%)	204/224 (91.1%)	221/224 (98.7%)	120/223 (53.8%)	56/110 (50.9%)	64/113 (56.6%)
W16	197/197 (100%)	193/196 (98.5%)	116/197 (58.9%)	171/196 (87.2%)	194/196 (99.0%)	129/195 (66.2%)	66/96 (68.8%)	63/99 (63.6%)
W20	168/168 (100%)	155/165 (93.9%)	80/168 (47.6%)	133/165 (80.6%)	164/165 (99.4%)	121/168 (72.0%)	62/82 (75.6%)	59/86 (68.6%)
W24	145/145 (100%)	129/143 (90.2%)	57/145 (39.3%)	103/143 (72.0%)	143/143 (100%)	107/144 (74.3%)	24 58/71 (81.7%)	49/73 (67.1%)
W28	131/131 (100%)	112/130 (86.2%)	45/131 (34.4%)	82/130 (63.1%)	129/130 (99.2%)	93/130 (71.5%)	52/68 (76.5%)	41/62 (66.1%)
W32	108/108 (100%)	89/106 (84.0%)	33/108 (30.6%)	62/106 (58.5%)	106/106 (100%)	77/108 (71.3%)	45/60 (75.0%)	32/48 (66.7%)
W36	83/83 (100%)	69/83 (83.1%)	25/83 (30.1%)	51/83 (61.4%)	83/83 (100%)	64/83 (77.1%)	39/46 (84.8%)	25/37 (67.6%)
Entire Study	255/261 (97.7%)	258/261 (98.9%)	259/261 (99.2%)	258/261 (98.9%)	260/261 (99.6%)	194/261 (74.3%)	104/131 (79.4%)	90/130 (69.2%)

(Source of data: adopted from Table 14.3.6.3.2 of 165-301 CSR)

Table 17. Anti-PAL IgG and Anti-PAL IgM antibody titers over time for the Phase 3 Population duration the first 36 weeks after pegvaliase administration

Study Week	Anti-PAL IgG			Anti-PAL IgM		
	20 mg/day (N = 131)	40 mg/day (N = 130)	Total (N = 261)	20 mg/day (N = 131)	40 mg/day (N = 130)	Total (N = 261)
Baseline						
N	129	130	259	130	129	259
Mean (SD)	7 (44)	111 (1,076)	59 (763)	185 (266)	304 (779)	244 (583)
Median	0	0	0	127	138	131
Min, Max	0 , 450	0 , 12,150	0 , 12,150	0 , 2,116	0 , 8,201	0 , 8,201
W3						
N	129	126	255	129	126	255
Mean (SD)	91 (506)	28 (166)	60 (379)	897 (3,564)	550 (955)	725 (2,623)
Median	0	0	0	243	323	296
Min, Max	0 , 5,400	0 , 1,800	0 , 5,400	0 , 38,040	36 , 7,654	0 , 38,040
W4						
N	128	129	257	128	129	257
Mean (SD)	305 (1,582)	33 (133)	169 (1,126)	527 (1,096)	582 (1,135)	555 (1,114)
Median	0	0	0	174	230	199
Min, Max	0 , 12,150	0 , 1,350	0 , 12,150	0 , 9,666	32 , 8,143	0 , 9,666
W8						
N	126	125	251	127	125	252
Mean (SD)	21,996 (53,904)	30,289 (68,259)	26,126 (61,491)	2,020 (2,863)	3,301 (5,081)	2,655 (4,157)
Median	4,050	4,050	4,050	1,034	1,452	1,272
Min, Max	0 , 328,050	0 , 328,050	0 , 328,050	39 , 22,050	89 , 26,970	39 , 26,970
W12						
N	111	111	225	111	113	224
Mean (SD)	530,509 (801,300)	622,943 (1,107,504)	577,343 (967,580)	2,474 (4,791)	4,141 (13,006)	3,315 (9,848)
Median	328,050	328,050	328,050	836	1,132	1,006
Min, Max	450 , 2,952,450	0 , 8,857,350	0 , 8,857,350	126 , 36,730	115 , 129,900	115 , 129,900
W16						
N	96	101	197	96	100	196
Mean (SD)	950,194 (1,645,572)	874,736 (1,240,573)	911,507 (1,448,845)	1,526 (2,814)	1,526 (3,332)	1,526 (3,081)
Median	328,050	328,050	328,050	595	624	609
Min, Max	450 , 8,857,350	150 , 8,857,350	150 , 8,857,350	115 , 22,700	106 , 31,590	106 , 31,590
W20						
N	82	86	168	80	85	165
Mean (SD)	817,573 (921,626)	1,005,712 (1,329,190)	913,882 (1,148,965)	1,078 (1,941)	838 (881)	955 (1,492)
Median	328,050	656,100	328,050	517	550	525
Min, Max	1,350 , 2,952,450	150 , 8,857,350	150 , 8,857,350	37 , 10,390	63 , 6,654	37 , 10,390
W24						

N	72	73	145	71	72	143
Mean (SD)	854,588 (1,493,932)	920,694 (1,023,666)	887,869 (1,274,937)	1,029 (3,470)	829 (1,089)	928 (2,557)
Median	328,050	328,050	328,050	413	524	466
Min, Max	1,350 , 8,857,350	450 , 2,952,450	450 , 8,857,350	35 , 28,450	96 , 6,149	35 , 28,450
W28						
N	68	63	131	67	63	130
Mean (SD)	975,613 (1,324,701)	1,003,650 (1,105,789)	989,097 (1,219,745)	793 (2,216)	852 (1,532)	822 (1,908)
Median	656,100	328,050	328,050	323	473	423
Min, Max	1,350 , 8,857,350	450 , 2,952,450	450 , 8,857,350	35 , 17,100	58 , 9,482	35 , 17,100
W32						
N	60	48	108	59	47	106
Mean (SD)	1,057,950 (1,355,746)	1,464,600 (1,947,214)	1,238,683 (1,649,304)	922 (3,797)	775 (1,230)	857 (2,938)
Median	984,150	984,150	984,150	337	494	423
Min, Max	1,350 , 8,857,350	450 , 8,857,350	450 , 8,857,350	21 , 29,400	30 , 8,060	21 , 29,400
W36						
N	46	37	83	46	37	83
Mean (SD)	1,003,461 (976,927)	1,575,061 (2,118,638)	1,258,270 (1,605,014)	1,183 (5,794)	1,323 (5,094)	1,245 (5,461)
Median	984,150	984,150	984,150	216	408	318
Min, Max	1,350 , 2,952,450	450 , 8,857,350	450 , 8,857,350	16 , 39,560	10 , 31,320	10 , 39,560
Entire Study						
N	131	130	261	131	130	261
Mean (SD)	429,402 (980,414)	486,431 (1,069,416)	457,863 (1,025,955)	1,139 (3,118)	1,435 (5,013)	1,286 (4,173)
Median	36,450	12,150	36,450	445	503	483
Min, Max	0 , 8,857,350	0 , 8,857,350	0 , 8,857,350	0 , 39,560	0 , 129,900	0 , 129,900

(Source of data: adopted from Table 14.3.6.3.3 of 165-301 CSR)

Table 18. Anti-PEG IgG and Anti-PEG IgM antibody titers over time for the Phase 3 Population duration the first 36 weeks after pegvaliase administration

Study Week	Anti-PEG IgG			Anti-PEG IgM		
	20 mg/day (N = 131)	40 mg/day (N = 130)	Total (N = 261)	20 mg/day (N = 131)	40 mg/day (N = 130)	Total (N = 261)
Baseline						
N	129	130	259	130	129	259
Mean (SD)	29 (86)	44 (144)	36 (119)	211 (1,398)	144 (364)	178 (1,022)
Median	10	10	10	0	0	0
Min, Max	0 , 810	0 , 810	0 , 810	0 , 15,300	0 , 2,404	0 , 15,300
W3						
N	129	126	255	129	126	255
Mean (SD)	9,142 (27,814)	6,714 (13,983)	7,942 (22,081)	23,594 (109,107)	19,878 (54,557)	21,758 (86,414)
Median	270	810	270	639	1,681	962
Min, Max	0 , 196,830	0 , 65,610	0 , 196,830	0 , 1,157,000	0 , 383,200	0 , 1,157,000
W4						
N	128	128	256	128	129	257
Mean (SD)	4,619 (12,293)	4,134 (8,442)	4,376 (10,527)	14,668 (72,747)	15,201 (49,897)	14,935 (62,212)
Median	90	270	270	547	1,266	634
Min, Max	0 , 65,610	0 , 65,610	0 , 65,610	0 , 799,400	0 , 407,000	0 , 799,400
W8						
N	126	125	251	127	125	252
Mean (SD)	2,319 (7,491)	1,240 (3,265)	1,782 (5,800)	26,046 (50,849)	31,834 (54,955)	28,917 (52,899)
Median	90	90	90	6,730	7,040	6,956
Min, Max	0 , 65,610	0 , 21,870	0 , 65,610	0 , 313,000	0 , 300,000	0 , 313,000
W12						
N	111	114	225	111	113	224
Mean (SD)	271 (1,026)	371 (2,093)	321 (1,652)	32,625 (127,570)	62,346 (505,992)	47,618 (369,916)
Median	30	30	30	531	595	575
Min, Max	0 , 7,290	0 , 21,870	0 , 21,870	0 , 1,160,000	0 , 5,360,000	0 , 5,360,000
W16						
N	96	101	197	96	100	196
Mean (SD)	202 (1,045)	47 (106)	123 (736)	11,261 (63,396)	4,690 (20,303)	7,908 (46,670)
Median	10	10	10	251	295	271
Min, Max	0 , 7,290	0 , 810	0 , 7,290	0 , 603,000	0 , 182,000	0 , 603,000
W20						
N	82	86	168	80	85	165
Mean (SD)	160 (850)	46 (151)	102 (604)	13,096 (61,677)	397 (926)	6,554 (43,283)
Median	0	0	0	145	124	129
Min, Max	0 , 7,290	0 , 810	0 , 7,290	0 , 453,200	0 , 7,880	0 , 453,200
W24						

N	72	73	145	71	72	143
Mean (SD)	137 (862)	31 (108)	84 (613)	7,380 (51,455)	409 (1,204)	3,870 (36,306)
Median	0	0	0	82	95	88
Min, Max	0 , 7,290	0 , 810	0 , 7,290	0 , 432,500	0 , 9,220	0 , 432,500
W28						
N	68	63	131	67	63	130
Mean (SD)	53 (297)	126 (917)	88 (669)	1,027 (4,505)	251 (476)	651 (3,262)
Median	0	0	0	78	38	55
Min, Max	0 , 2,430	0 , 7,290	0 , 7,290	0 , 34,200	0 , 2,360	0 , 34,200
W32						
N	60	48	108	59	47	106
Mean (SD)	57 (316)	29 (122))	45 (248	315 (944)	252 (501)	287 (777)
Median	0	0	0	45	37	40
Min, Max	0 , 2,430	0 , 810	0 , 2,430	0 , 6,530	0 , 2,650	0 , 6,530
W36						
N	46	37	83	46	37	83
Mean (SD)	19 (57)	71 (399)	43 (269)	575 (1,994)	113 (191)	369 (1,501)
Median	0	0	0	62	48	50
Min, Max	0 , 270	0 , 2,430	0 , 2,430	0 , 9,900	0 , 621	0 , 9,900
Entire Study						
N	131	130	261	131	130	261
Mean (SD)	2,107 (11,313)	1,527 (6,230)	1,818 (9,140)	13,756 (70,400)	15,394 (168,420)	14,572 (128,900)
Median	10	10	10	212	281	242
Min, Max	0 , 196,830	0 , 65,610	0 , 196,830	0 , 1,160,000	0 , 5,360,000	0 , 5,360,000

(Source of data: adopted from Table 14.3.6.3.3 of 165-301 CSR)

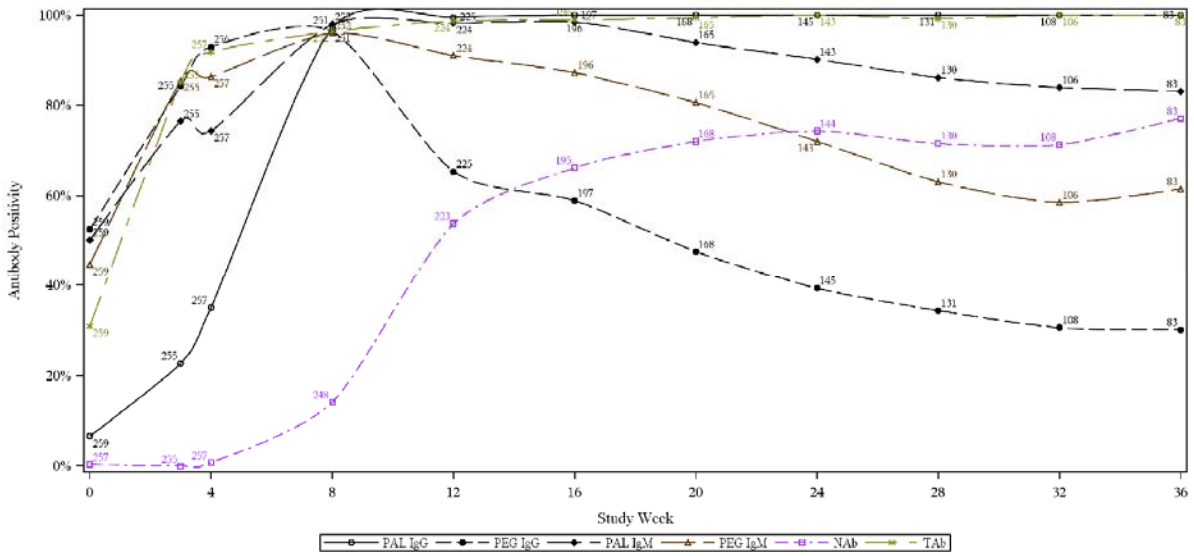
Table 19. TAb and Anti-PAL NAb antibody titers over time for the Phase 3 Population duration the first 36 weeks after pegvaliase administration

Study Week	TAb			NAb		
	20 mg/day (N = 131)	40 mg/day (N = 130)	Total (N = 261)	20 mg/day (N = 131)	40 mg/day (N = 130)	Total (N = 261)
Baseline						
N	130	129	259	128	129	257
Mean (SD)	238 (1063)	174 (827)	206 (951)	0 (0)	0 (2)	0 (1)
Median	0	0	0	0	0	0
Min, Max	0 , 10,700	0 , 8,550	0 , 10,700	0 , 1	0 , 18	0 , 18
W3						
N	129	126	255	129	126	255
Mean (SD)	74,764 (196,602)	59,328 (122,688)	67,137 (164,152)	0 (0)	0 (0)	0 (0)
Median	3,490	11,600	7,570	0	0	0
Min, Max	0 , 1,060,000	0 , 850,000	0 , 1,060,000	0 , 1	0 , 1	0 , 1
W4						
N	128	129	257	128	129	257
Mean (SD)	43,626 (129,214)	28,480 (58,633)	36,023 (100,296)	0 (0)	0 (2)	0 (1)
Median	1,745	6,600	3,800	0	0	0
Min, Max	0 , 928,000	0 , 441,000	0 , 928,000	0 , 2	0 , 18	0 , 18
W8						
N	127	125	252	125	123	248
Mean (SD)	39,841 (189,267)	17,839 (34,345)	28,928 (136,699)	5 (44)	12 (64)	8 (55)
Median	1,790	3,450	3,330	0	0	0
Min, Max	0 , 2,020,000	0 , 281,000	0 , 2,020,000	0 , 486	0 , 486	0 , 486
W12						
N	111	113	224	110	113	223
Mean (SD)	13,204 (34,115)	15,477 (44,193)	14,350 (39,449)	219 (544)	270 (463)	245 (504)
Median	1,560	2,650	2,130	2	18	6
Min, Max	0 , 295,000	0 , 314,000	0 , 314,000	0 , 4,374	0 , 1,458	0 , 4,374
W16						
N	96	100	196	96	99	195
Mean (SD)	24,408 (152,823)	11,099 (17,823)	17,618 (107,628)	685 (4,012)	399 (739)	540 (2,860)
Median	3,090	3,860	3,420	108	54	54
Min, Max	0 , 1,500,000	0 , 104,000	0 , 1,500,000	0 , 39,366	0 , 4,374	0 , 39,366
W20						
N	80	85	165	82	86	168
Mean (SD)	13,255 (20,718)	13,894 (20,042)	13,584 (20,313)	359 (647)	634 (1,000)	500 (855)
Median	3,955	4,010	4,010	54	162	162
Min, Max	112 , 107,000	0 , 87,600	0 , 107,000	0 , 4,374	0 , 4,374	0 , 4,374
W24						

N	71	72	143	71	73	144
Mean (SD)	12,643 (19,974)	17,800 (26,826)	15,239 (23,731)	340 (466)	404 (511)	372 (489)
Median	3,610	5,215	3,980	162	162	162
Min, Max	62 , 99,500	314 , 112,000	62 , 112,000	0 , 1,458	0 , 1,458	0 , 1,458
W28						
N	67	63	130	68	62	130
Mean (SD)	14,960 (21,174)	21,558 (28,889)	18,158 (25,327)	446 (701)	426 (544)	436 (629)
Median	7,870	7,860	7,865	162	162	162
Min, Max	232 , 91,200	0 , 109,000	0 , 109,000	0 , 4,374	0 , 1,458	0 , 4,374
W32						
N	59	47	106	60	48	108
Mean (SD)	17,978 (25,245)	23,172 (29,670)	20,281 (27,284)	456 (862)	757 (1,918)	590 (1,431)
Median	7,650	10,200	9,075	162	324	162
Min, Max	553 , 102,000	700 , 109,000	553 , 109,000	0 , 4,374	0 , 13,122	0 , 13,122
W36						
N	46	37	83	46	37	83
Mean (SD)	15,836 (17,989)	25,557 (32,617)	20,169 (25,851)	381 (460)	668 (1,062)	509 (795)
Median	9,980	10,300	10,200	162	162	162
Min, Max	430 , 69,100	50 , 107,000	50 , 107,000	0 , 1,458	0 , 4,374	0 , 4,374
Entire Study						
N	131	130	261	131	130	261
Mean (SD)	27,424 (116,777)	21,069 (54,980)	24,257 (91,397)	206 (1,280)	234 (677)	220 (1,025)
Median	2,850	3,630	3,260	0	0	0
Min, Max	0 , 2,020,000	0 , 850,000	0 , 2,020,000	0 , 39,366	0 , 13,122	0 , 39,366

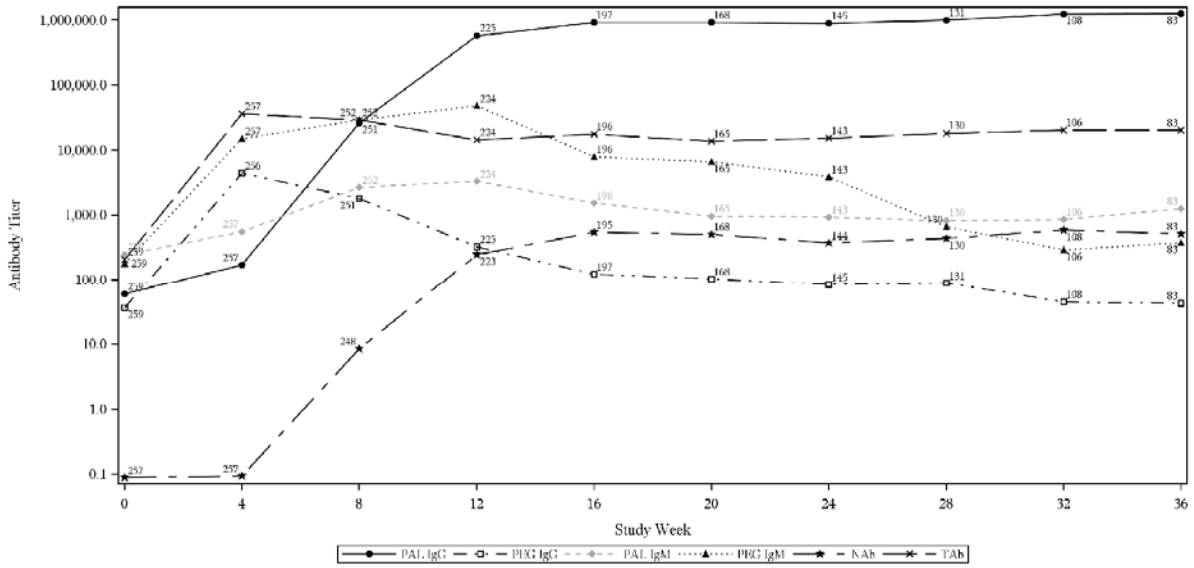
(Source of data: adopted from Table 14.3.6.3.3 of 165-301 CSR)

Figure 17. Incidence of antibody positivity for the Phase 3 Population during the first 36 weeks after pegvaliase administration



(Source of data: Figure 12.2.1.1.1 of 165-301 CSR)

Figure 18. Mean antibody titers over time for the Phase 3 Population duration the first 36 weeks after pegvaliase administration



(Source of data: Figure 14.3.6.3.2 of 165-301 CSR)

1.4.4 Anti-Drug Antibody Responses in Study 165-302

Study 165-302 was a four-part, Phase 3 study with adult PKU patients who completed a previous pegvaliase study with daily SC injection of pegvaliase (i.e., PAL-003, 165-205, or 165-301).

A total of 215 subjects enrolled into 165-302. Most of the subjects (n = 203) were from 165-301 in which pegvaliase was administered as I/T/M regimen; the remaining 12 subjects were enrolled from PAL-003 (adjustable pegvaliase dosing up to 60 mg/day) or 165-205 (pegvaliase administered as I/T/M regimen). Pegvaliase dosing was continued without interruption from the previous study until Day 1 of 165-302. Because most of the subjects in 165-302 were from 165-301, the immunogenicity data from165-302 represented a continuum of the data from 165-301 after the Maintenance Period. The 12 subjects transitioned from Phase 2 studies (i.e., 165-205 and PAL 003) entered 165-302 at Week 24 to Week 276 after initial administration of pegvaliase.

Anti-drug antibodies were detected once in > 95% of the subjects for anti-PAL IgG, anti-PAL IgM, TAb, and NAb, whereas anti-PEG IgG and anti-PEG IgM antibodies were tested positive at least once in 50.7% and 90.7% of subjects, respectively (**Table 20**). Similar incidence of antibody positivity was observed throughout 165-302 for anti-PAL IgG, anti-PAL IgM, TAb, and NAb (**Table 20**). However, the incidence of anti-PEG IgG decreased from 29% in Part 1 Week 1 (i.e., end of 165-301) to 18.5% in Part 1 Week 13; similar decline in the incidence of anti-PEG IgG positivity was also observed in Part 4. A slight decline in the incidence of anti-PEG IgM positivity over time was observed in Part 1 and Part 4.

Overall, mean antibody titers for anti-PAL IgG, anti-PAL IgM, TAb, and NAb remained relatively stable in all subjects in 165-302 **(Figure 19**) after the initial antibody response in 165-301 (see Section 1.4.3 above). On the other hand, mean titers for anti-PEG IgG and anti-PEG IgM seemed to decrease below baseline values over time in Part 4 (**Table 21**), potentially due to drug interference from the anti-PEG antibody assays. See Section 1.1.3.1 for more information.

Table 20. Incidences of antidrug antibodies for the Phase 3 Population in Parts 1 to 4 of 165-302

	Anti-PAL IgG	Anti-PAL IgM	Anti-PEG IgG	Anti-PEG IgM	TAb	NAb
Part 1 (N = 164)						
W1	162/162 (100%)	148/162 (91.4%)	47/162 (29.0%)	110/162 (67.9%)	162/162 (100%)	124/162 (76.5%)
W5	99/99 (100%)	88/100 (88.0%)	28/99 (28.3%)	72/100 (72.0%)	100/100 (100%)	76/99 (76.8%)
W9	65/65 (100%)	49/65 (75.4%)	16/65 (24.6%)	43/65 (66.2%)	65/65 (100%)	52/65 (80.0%)
W13	27/27 (100%)	22/26 (84.6%)	5/27 (18.5%)	18/26 (69.2%)	26/26 (100%)	20/27 (74.1%)
W17	17/17 (100%)	12/17 (70.6%)	2/17 (11.8%)	7/17 (41.2%)	17/17 (100%)	13/17 (76.5%)
W21	15/15 (100%)	14/15 (93.3%)	2/15 (13.3%)	8/15 (53.3%)	15/15 (100%)	10/15 (66.7%)
W25	13/13 (100%)	11/13 (84.6%)	1/13 (7.7%)	7/13 (53.8%)	13/13 (100%)	7/13 (53.8%)
W29	7/7 (100%)	6/7 (85.7%)	0/7 (0%)	5/7 (71.4%	7/7 (100%)	5/7 (71.4%)
W33	2/2 (100%)	2/2 (100%)	0/2 (0%)	0/2 (0.0%)	2/2 (100%)	1/2 (50.0%)
W37	1/1 (100%)	1/1 (100%)	0/1 (0%)	0/1 (0.0%)	1/1 (100%)	1/1 (100%)
Part 2 (N = 66)*						
Baseline	66/66 (100%)	57/66 (86.1%)	8/66 (12.1%)	36/66 (54.5%)	66/66 (100%)	44/66 (66.7%)
W4	45/45 (100%)	38/45 (84.4%)	5/45 (11.1%)	26/45 (57.8%)	45/45 (100%)	29/45 (64.4%)
W8	58/58 (100%)	51/58 (87.9%)	6/58 (10.3%)	33/58 (56.9%)	58/58 (100%)	38/58 (65.5%)
Part 3 (N = 41)*						
W4	41/41 (100%)	33/41 (80.5%)	3/41 (7.3%)	26/41 (63.4%)	41/41 (100%)	24/41 (58.5%)
Part 4 (N = 202)						
W1	184/184 (100%)	164/184 (89.1%)	62/184 (33.7%)	125/184 (67.9%)	183/184 (99.5%)	145/184 (78.8%)
W5	165/165 (100%)	155/165 (93.9%)	31/164 (18.9%)	119/165 (72.1%)	163/165 (98.8%)	123/165 (74.5%)
W9	196/196 (100%)	185/197 (93.9%)	31/196 (15.8%)	119/197 (60.4%)	197/197 (100%)	154/195 (79.0%)
W13	168/168 (100%)	150/167 (89.8%)	21/168 (12.5%)	108/167 (64.7%)	166/167 (99.4%)	131/168 (78.0%)
W25	165/165 (100%)	145/166 (87.3%)	11/163 (6.7%)	98/166 (59.0%)	165/166 (99.4%)	130/165 (78.8%)
W49	109/109 (100%)	99/109 (90.8%)	2/106 (1.9%)	53/109 (48.6%)	108/108 (100%)	86/109 (78.9%)
W73	91/91 (100%)	76/91 (83.5%)	3/75 (4.0%)	41/91 (45.1%)	89/91 (97.8%)	70/91 (76.9%)
W97	40/40 (100%)	32/39 (82.1%)	3/30 (10.0%)	18/40 (45.0%)	39/40 (97.5%)	33/40 (82.5%)
W121	28/28 (100%)	21/28 (75.0%)	0/14 (0%)	11/28 (39.3%)	27/28 (96.4%)	21/28 (75.0%)
W145	4/4 (100%)	2/4 (50.0%)	0/1 (0%)	0/4 (0%)	3/4 (75.0%)	2/4 (50.0%)
Entire Study (N = 215)	215/215 (100%)	213/215 (99.1%)	109/215 (50.7%)	195/215 (90.7%)	215/215 (100%)	206/215 (95.8%)

*Subjects randomized to the placebo group were excluded

(Source of data: Representative data from Tables 14.3.6.68 to 14.3.6.72 of 165-302 CSR from the initial submission)

Table 21. Antidrug antibody titers for the Phase 3 Population in Parts 1 to 4 of 165-302

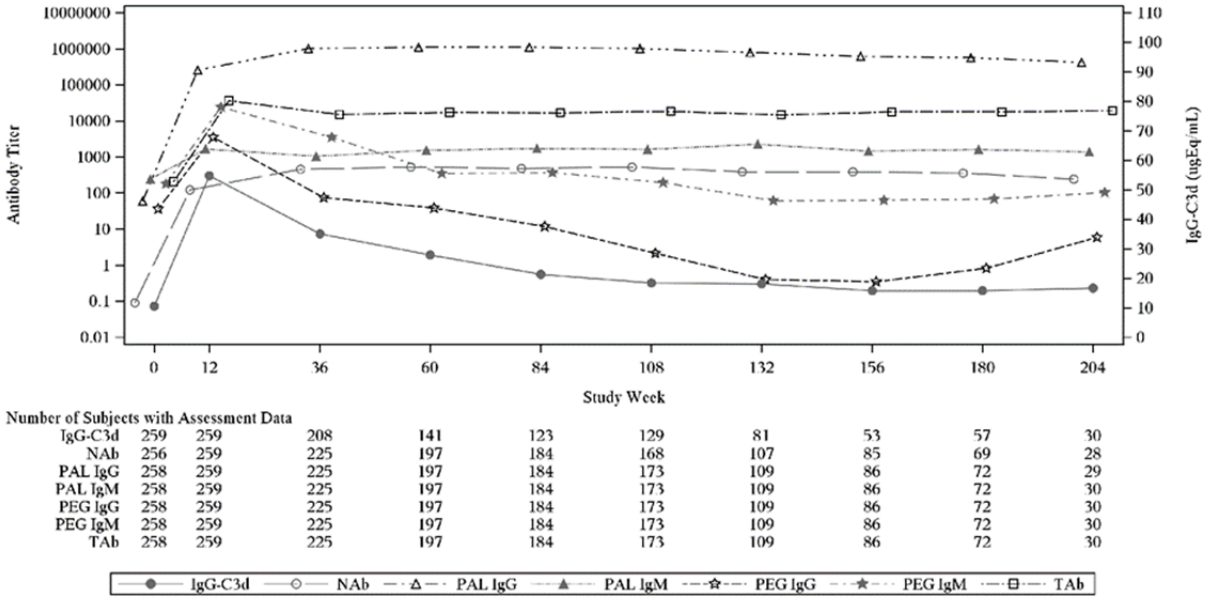
Study Week	Anti-PAL IgG	Anti-PAL IgM	Anti-PEG IgG	Anti-PEG IgM	TAb	NAb
Part 1 (N = 164)						
W1						
N	162	162	162	162	162	162
Mean (SD)	920,567 (1,222,072)	1,206 (5,078)	76 (605)	300 (903)	17,659 (28,287)	456 (684)
Median	328,050	454	0	69	7,945	162
Min, Max	1,350 , 8,857,350	0 , 51,200	0 , 7,290	0 , 9,240	296 , 214,000	0 , 4,374
W5						
N	99	100	99	100	100	99
Mean (SD)	1,144,186 (1,304,625)	2,309 (13,122)	116 (773)	316 (791)	22,200 (28,595)	591 (722)
Median	984,150	409	0	107	9,475	162
Min, Max	12,150 , 8,857,350	0 , 113,000	0 , 7,290	0 , 7,060	239 , 107,000	0 , 4,374
W9						
N	65	65	65	65	65	65
Mean (SD)	1,374,445 (1,686,597)	792 (3,989)	85 (423)	495 (2,270)	23,762 (27,386)	968 (2,344)
Median	984,150	208	0	77	11,100	486
Min, Max	36,450 , 8,857,350	0 , 32,400	0 , 2,430	0 , 18,300	859 , 109,000	0 , 13,122
Entire Part 1						
N	164	164	164	164	164	164
Mean (SD)	965,616 (1,087,798)	1,412 (5,797)	68 (491)	320 (966)	18,603 (27,366)	524 (779)
Median	656,100	418	0	88	8,251	162
Min, Max	1,350 , 6,889,050	0 , 50,867	0 , 5,670	0 , 11,063	296 , 214,000	0 , 5,346
Part 2 (N = 66)						
Baseline						
N	66	66	66	66	66	66
Mean (SD)	624,273 (688,279)	423 (422)	4 (16)	101 (170)	13,213 (19,751)	237 (397)
Median	328,050	319	0	29	5,950	54
Min, Max	1,350 , 2,952,450	0 , 2,380	0 , 90	0 , 780	239 , 104,000	0 , 1,458
W4						
N	45	45	45	45	45	45
Mean (SD)	726,690 (941,385)	331 (390)	2 (5)	82 (190)	12,046 (17,238)	271 (708)
Median	328,050	172	0	28	7,340	18
Min, Max	1,350 , 2,952,450	0 , 2,270	0 , 30	0 , 1,210	191 , 106,000	0 , 4,374
W8						
N	58	58	58	58	58	58
Mean (SD)	758,607 (1,300,480)	396 (478)	8 (37)	102 (272)	13,122 (16,563)	251 (381)
Median	328,050	215	0	27	7,650	162

Min, Max	4,050 , 8,857,350	0 , 2,520	0 , 270	0 , 1,873	242 , 87,200	0 , 1,458
Entire Part 2						
N	66	66	66	66	66	66
Mean (SD)	682,732 (899,660)	410 (431)	6 (20)	114 (215)	13,184 (16,767)	269 (436)
Median	328,050	266	0	39	6,830	99
Min, Max	1,350 , 5,904,900	0 , 2,450	0 , 135	0 , 1,268	247 , 92,075	0 , 2,430
Part 3 (N = 41)						
W4						
N	42	41	42	41	41	41
Mean (SD)	806,336 (875,383)	471 (488)	1 (3)	63 (79)	11,253 (10,089)	236 (436)
Median	328,050	340	0	32	7,160	18
Min, Max	4,050 , 2,952,450	0 , 2,316	0 , 10	0 , 306	702 , 38,600	0 , 1,458
Part 4 (N = 202)						
W1						
N	184	184	184	184	184	184
Mean (SD)	1,135,032 (1,497,098)	1,610 (6,769)	37 (201)	2,516 (18,454)	13,082 (17,806)	611 (1,515)
Median	984,150	426	0	65	8,110	162
Min, Max	450 , 8,857,350	0 , 73,900	0 , 2,430	0 , 212,000	0 , 107,000	0 , 13,122
W5						
N	165	165	164	165	165	165
Mean (SD)	1,305,421 (1,593,808)	1,786 (8,950)	31 (210)	1,720 (9,966)	14,159 (17,859)	489 (796)
Median	984,150	525	0	48	8,200	162
Min, Max	150 , 8,857,350	0 , 107,000	0 , 2,430	0 , 103,000	0 , 101,000	0 , 4,374
W9						
N	196	197	195	197	197	195
Mean (SD)	1,241,284 (1,567,599)	2,258 (13,468)	27 (193)	787 (4,188)	14,239 (18,167)	466 (1,041)
Median	984,150	457	0	38	8,640	162
Min, Max	1,350 , 8,857,350	26 , 137,000	0 , 2,430	0 , 39,900	159 , 102,000	0 , 13,122
W13						
N	168	167	168	167	167	168
Mean (SD)	1,290,432 (1,540,056)	1,647 (6,185)	13 (75)	849 (6,866)	13,857 (15,967)	521 (1,247)
Median	984,150	482	0	38	8,460	162
Min, Max	150 , 8,857,350	36 , 63,700	0 , 810	0 , 80,800	0 , 85,700	0 , 13,122
W25						
N	165	166	163	166	166	165
Mean (SD)	1,258,915 (1,678,120)	1,529 (10,320)	6 (64)	113 (271)	16,329 (19,525)	452 (543)
Median	984,150	452	0	38	9,720	162
Min, Max	450 , 8,857,350	43 , 132,900	0 , 810	0 , 2,233	0 , 100,000	0 , 1,458
W49						
N	109	109	106	109	108	109

Mean (SD)	966,142 (1,015,380)	1,723 (6,671)	0 (1)	50 (86)	14,628 (19,002)	497 (823)
Median	984,150	406	0	0	8,420	162
Min, Max	1,350 , 2,952,450	46 , 56,020	0 , 10	0 , 485	85 , 101,000	0 , 4,374
W73						
N	91	91	75	91	91	91
Mean (SD)	804,555 (918,039)	2,714 (10,762)	11 (94)	62 (173)	18,924 (21,606)	442 (760)
Median	328,050	438	0	0	10,900	162
Min, Max	1,350 , 2,952,450	33 , 90,650	0 , 810	0 , 1,380	0 , 92,300	0 , 4,374
W97						
N	40	39	30	40	40	40
Mean (SD)	827,145 (973,151)	1,323 (4,037)	1 (3)	95 (283)	14,901 (21,505)	496 (592)
Median	328,050	323	0	0	9,630	162
Min, Max	1,350 , 2,952,450	14 , 24,360	0 , 10	0 , 1,714	0 , 109,000	0 , 1,458
W121						
N	28	28	14	28	28	28
Mean (SD)	579,600 (749,805)	758 (1,734)	0 (0)	204 (580)	12,989 (14,726)	360 (441)
Median	328,050	302	0	0	6,755	162
Min, Max	450 , 2,952,450	33 , 8,865	0 , 0	0 , 2,220	0 , 69,500	1 , 1,458
W145						
N	4	4	1	4	4	4
Mean (SD)	94,275 (156,570)	124 (59)	0	0	4,095 (5,026)	123 (242)
Median	24,300	133	0	0	2,640	2
Min, Max	450 , 328,050	46 , 183	0	0	0 , 11,100	1 , 486
Entire Study						
N	215	215	215	215	215	215
Mean (SD)	1,109,811 (1,128,182)	1,700 (7,456)	40.4 (255)	1,061 (6,846)	16,291 (18,202)	511 (613)
Median	820,125.0	462	0.5	69.1	10,069	287
Min, Max	641, 6,889,050	27, 83,607	0, 2,970	0, 90,700	153, 100,557	0, 3,402

(Source of data: Representative data from Tables 14.3.6.73 to 14.3.6.77 of 165-302 CSR)

Figure 19. Antidrug antibody titers and IgG-C3d CIC levels over time for the Phase 3 Population



(Source of data: Figure 13.3.1.23 of Response to Clinical Pharmacology Information Request dated 19January2018; SDN 47)

Reviewer's Comments

In pegvaliase Phase 3 clinical trials, declines in the incidences and mean titer values of anti-PEG IgG and IgM were observed over time. The incidence decreased to 0% for anti-PEG IgG and below the baseline incidence for anti-PEG IgM; the mean titer values dropped below the baseline titers values for both anti-PEG IgM and IgG. These declines are likely attributable to the poor drug tolerance of the two anti-PEG ADA assays. Hence, the anti-PEG IgG and IgM study results should be interpreted with caution. Please refer to Section 1.1.3.1 for discussion regarding the impact of pegvaliase concentration on the incidences and mean antibody titers of anti-PEG ADAs.

*The declines in anti-PEG ADAs were unlikely to be resulted from high dose immune tolerance due to the following reasons. First, the incidences and mean titer values of anti-PEG ADAs in the placebo group rebounded after pegvaliase was discontinued during the randomized discontinuation period (i.e., Part 2) of 165-302 (**Figure 4**), showing that the body was still able to produce anti-PEG antibodies. Second and more importantly, the incidences and mean titers of anti-PEG ADAs further decreased from the rebound after pegvaliase was reintroduced and concentration increased in 165-302 Part 3, demonstrating that the changes in incidences and mean titers are likely due to assay interference from pegvaliase present in the study samples.*

1.4.5 Anti-Pegvaliase IgG4 Responses in Phase 3 Studies

Majority of subjects developed anti-pegvaliase IgG4 responses with an overall incidence of 98.2 % (268 out of 273) and these responses were sustained over long term treatment (**Table 22**).

At baseline, 12.6% of the subjects were tested positive for anti-pegvaliase IgG4, and more than 90% of subjects were tested positive for anti-pegvaliase IgG4 from Weeks 12 – 168.

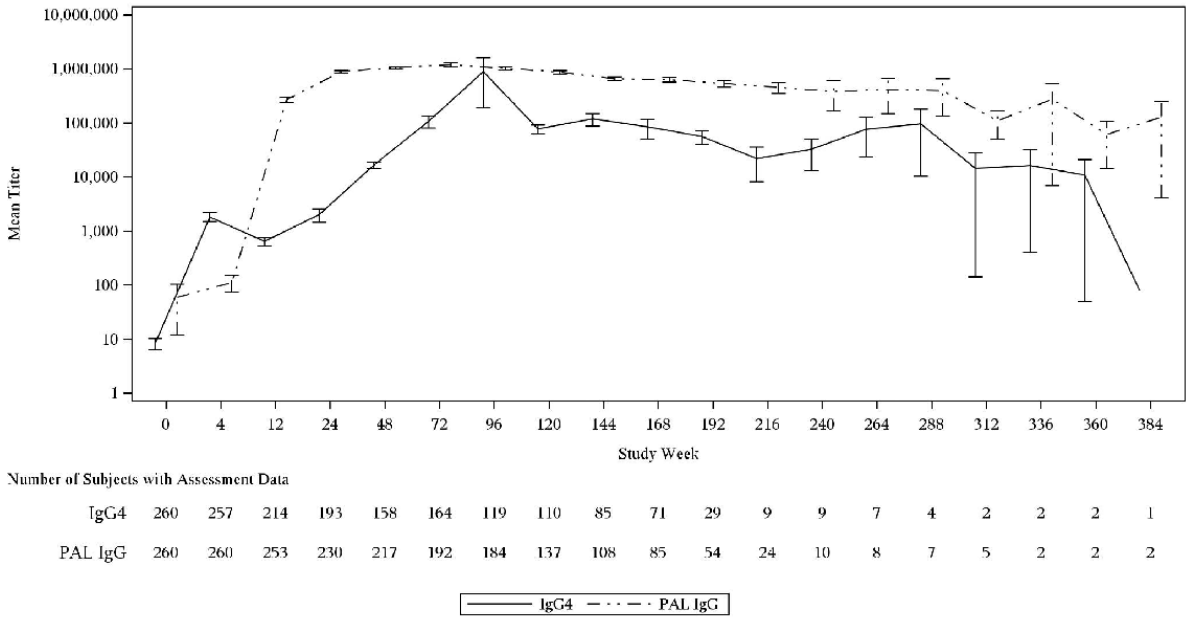
Mean anti-pegvaliase IgG4 titer at baseline in all subjects was 8 (range: 0 – 435; n = 260), increased to 1,823 (range: 0 – 79,900; n = 257) at Week 4 and reached its peak at Week 96 (Mean titer = 892,158, [range: 0 – 83,900,000]; n = 119) (**Table 22**). Anti-pegvaliase IgG4 titers were generally sustained over long term treatment with a mean titer of 84,652 (range: 0 – 2,430,000, n = 71) at Week 168 in all subjects. In contrast, mean anti-PAL IgG titer peaked much earlier (Week 12 – Week 16) and remained generally stable after that time point (**Figure 20**).

Table 22. Incidence of anti-pegvaliase IgG4 antibody positivity and anti-pegvaliase IgG4 titers over time for the Phase 3 Population

	Incidence	IgG4 Titers		
Study Visit	N (%)	Mean (SD)	Median	Min, Max
Baseline	33/261 (12.6 %)	8 (34)	0	0, 435
W4	206/257 (80.2 %)	1,823 (5,973)	229	0 , 79,9000
W12	201/214 (93.9 %)	647 (1,907)	235	0 , 22,100
W24	185/193 (95.9 %)	2,014 (8,289)	516	0 , 112,000
W48	154/158 (97.5 %)	16,678 (33,252)	5,070	0 , 272,000
W72	163/164 (99.4 %)	108,017 (367,393)	17,050	0 , 3,680,000
W96	115/119 (96.6 %)	892,158 (7,735,435)	35,500	0 , 83,900,000
W120	107/110 (97.3 %)	77,647 (163,575)	28,250	0 , 1,170,000
W144	81/ 85 (95.3 %)	119,134 (295,344)	28,100	0 , 2,350,000
W168	66/ 71 (93.0 %)	84,652 (295,461)	14,200	0 , 2,430,000
W192	26/ 29 (89.7 %)	55,992 (87,438)	18,300	0 , 316,000
W216	7/ 9 (77.8 %)	22,042 (42,127)	4,250	0 , 123,000
W240	7/ 9 (77.8 %)	32,693 (58,978)	2,660	0 , 176,000
W264	6/ 7 (85.7 %)	76,339 (140,727)	8,180	0 , 381,000
W288	4/ 4 (100 %)	96,554 (172,677)	15,595	25 , 355,000
W312	2/ 2 (100 %)	14,370 (20,124)	14,370	140 , 28,600
W336	2/ 2 (100 %)	16,499 (22,771)	16,499	397 , 32,600
W360	2/ 2 (100 %)	10,774 (15,168)	10,774	49 , 21,500
W384	1/ 1 (100 %)	81	81	81

(Source of data: Table 2.7.4.10.4 and 2.7.4.10.2.1 of 120-Safety Report Part 2; SDN 22)

Figure 20. Mean (SE) anti-pegvaliase IgG4 and anti-PAL IgG titers over time for the Phase 3 Population



SE, standard error; IgG, immunoglobulin G
The values at each time points are the average of all the values within each interval.

(Source of data: Figure 1.5.1 of IgG4 Report submitted in the 120-Safety Update; SDN 22)

1.4.6 Responses of CIC and Complement in Phase 3 Studies

Overall, mean IgG CIC and IgM CIC concentrations were highest at Week 12 during early treatment and returned towards baseline in longer-term treatment (**Table 23** and **Figure 21**). Because of the high degree of consistency in the pattern of IgG and IgM CIC development over time, the Applicant suggested that CICs are comprised of both IgM and IgG.

Mean complement C3 and C4 concentrations declined from baseline to reach nadir at 3 months and then slowly increased towards baseline levels (**Table 23**, **Figure 22**, and **Figure 23**). However, mean C3 concentration did not return to baseline level by 45 months after the initiation of pegvaliase (**Figure 22**). While mean C4 concentration returned to normal level at Month 45, there was wide variability at that time point (**Figure 23**).

The Applicant evaluated the changes from baseline of IgG/IgM CIC with those of time matched C3/C4 (**Figure 24**). Changes in IgG and IgM CIC concentrations from baseline were greatest during induction/titration when the C3/C4 concentrations were declining (time interval between Week 12 – Week 36). Mean IgG and IgM CIC concentrations declined as C3/C4 concentrations increased over time.

Table 23. CIC and complement concentrations over time for the Phase 3 Population

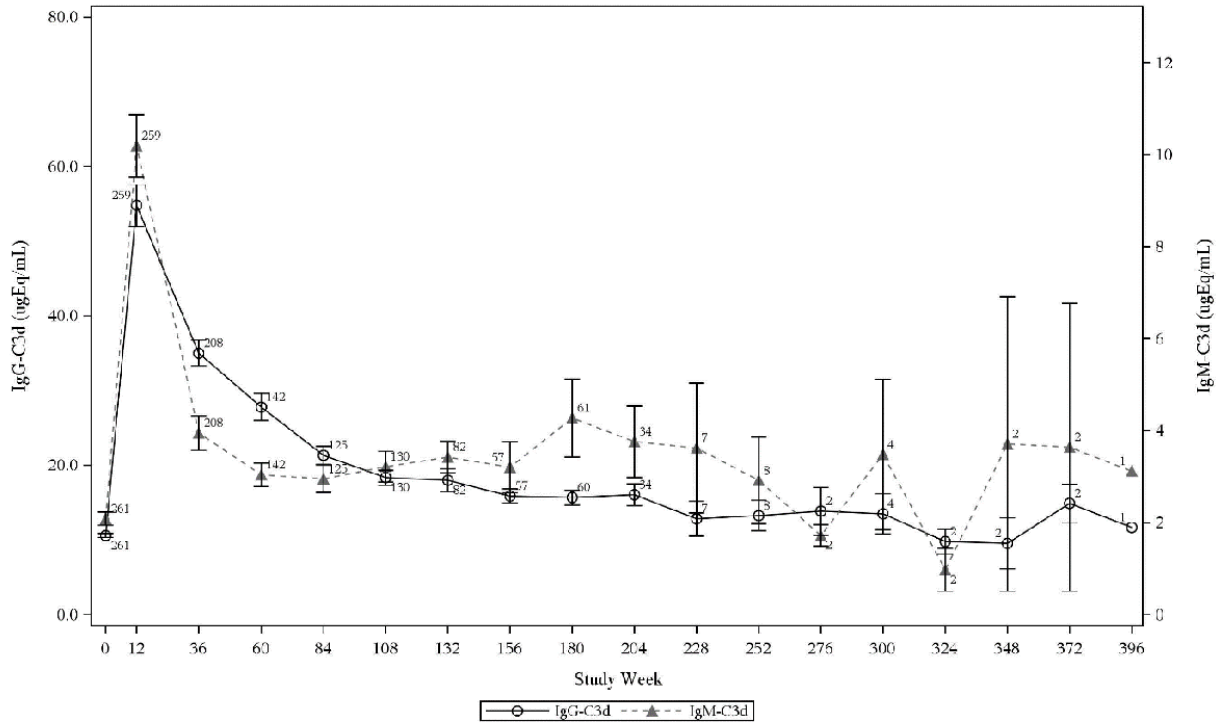
	IgG-C3d CIC (µgEq/mL) (N = 273)	IgM-C3d CIC (µgEq/mL) (N = 273)	C3 (g/L) (N = 259)	C4 (g/L) (N = 259)
Baseline				
N	261	261	257	257
Mean (SD)	10.59 (5.30)	2.09 (2.42)	1.19 (0.23)	0.24 (0.10)
Median	9.40	1.13	1.17	0.23
Min, Max	1.8, 37.1	0.5, 16.5	0.7, 2.2	0.1, 1.3
W12				
N	259	259	258	258
Mean (SD)	54.79 (46.13)	10.20 (11.02)	1.01 (0.29)	0.17 (0.08)
Median	41.88	5.94	0.97	0.16
Min, Max	3.0, 389.7	0.5, 70.8	0.4, 2.1	0.0, 0.6
W36				
N	208	208	226	226
Mean (SD)	35.01 (26.08)	3.95 (5.52)	0.83 (0.29)	0.16 (0.09)
Median	28.45	2.16	0.83	0.15
Min, Max	6.9, 140.4	0.5, 54.7	0.2, 1.8	0.0, 0.9
W60				
N	142	142	197	197
Mean (SD)	27.77 (22.17)	3.04 (3.16)	0.83 (0.30)	0.17 (0.09)
Median	23.25	2.08	0.85	0.16
Min, Max	4.9, 133.3	0.5, 22.3	0.2, 1.7	0.0, 0.8
W84				
N	120	120	155	155
Mean (SD)	21.41 (14.24)	2.94 (3.50)	0.86 (0.33)	0.18 (0.09)
Median	18.20	1.96	0.83	0.17
Min, Max	3.9, 101.1	0.5, 24.2	0.2, 2.1	0.0, 0.7
W108				
N	77	77	111	111
Mean (SD)	18.60 (10.45)	3.78 (4.52)	0.87 (0.30)	0.18 (0.07)
Median	15.00	2.45	0.89	0.19
Min, Max	3.6, 49.1	0.5, 27.8	0.2, 1.5	0.0, 0.5
W132				
N	61	61	80	80
Mean (SD)	18.27 (14.97)	3.14 (3.15)	0.91 (0.30)	0.19 (0.07)
Median	15.80	2.29	0.91	0.19
Min, Max	3.0, 112.1	0.5, 14.7	0.2, 1.6	0.0, 0.5
W156				
N	45	45	55	55
Mean (SD)	16.04 (7.23)	3.00 (4.58)	1.00 (0.30)	0.21 (0.06)
Median	14.60	2.29	1.03	0.21
Min, Max	4.3, 37.7	0.5, 30.0	0.4, 1.5	0.1, 0.4
W180				
N	20	20	18	18
Mean (SD)	15.91 (5.47)	3.75 (5.48)	1.06 (0.38)	0.23 (0.16)
Median	16.20	2.37	1.00	0.20
Min, Max	5.0, 28.8	0.5, 25.4	0.4, 2.0	0.1, 0.8
W204				
N	4	4	-	-

Mean (SD)	11.25 (5.88)	1.75 (1.65)		
Median	10.10	1.26		
Min, Max	5.6, 19.2	0.5, 4.0		

Data after Week 204 are not included in the table because of the small number of subject

(Source of data: Data combined from Tables 2.7.4.9.4.1, 2.7.4.9.4.2, 2.7.4.9.5.1, and 2.7.4.9.6.1 of ISS)

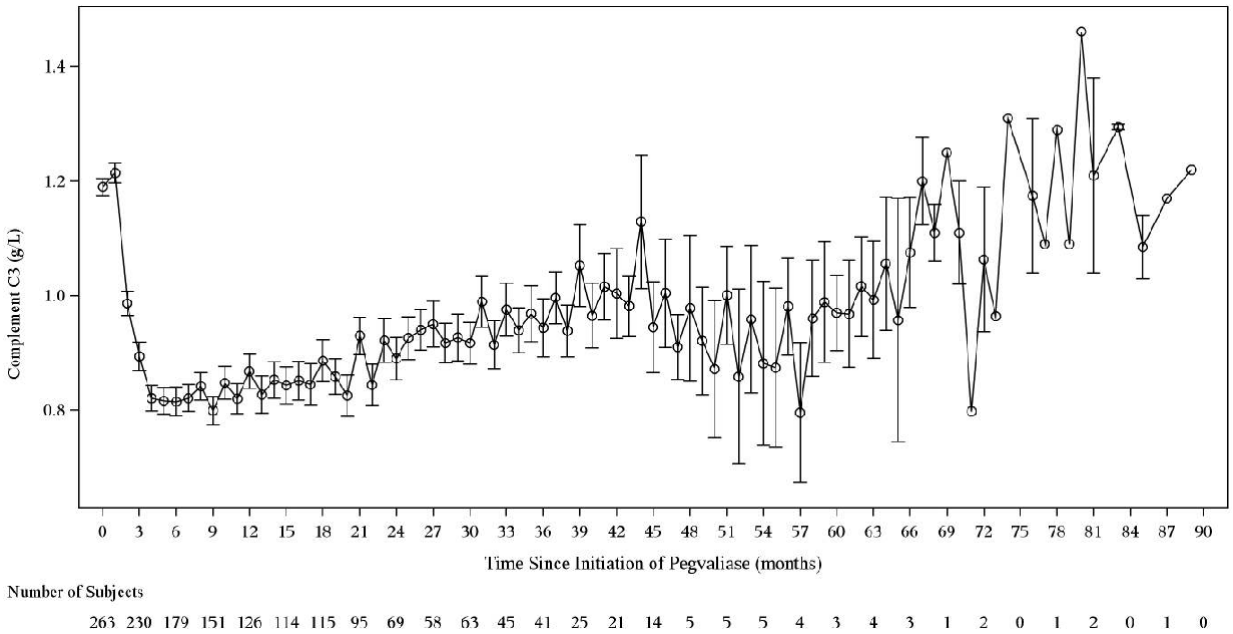
Figure 21. Mean (SE) IgG-C3d CIC and IgM-C3d CIC concentrations over time for the Phase 3 Population



CIC, circulating immune complex; Ig, immunoglobulin; SE, standard error.
The values at each time points are the average of all the values within each interval.

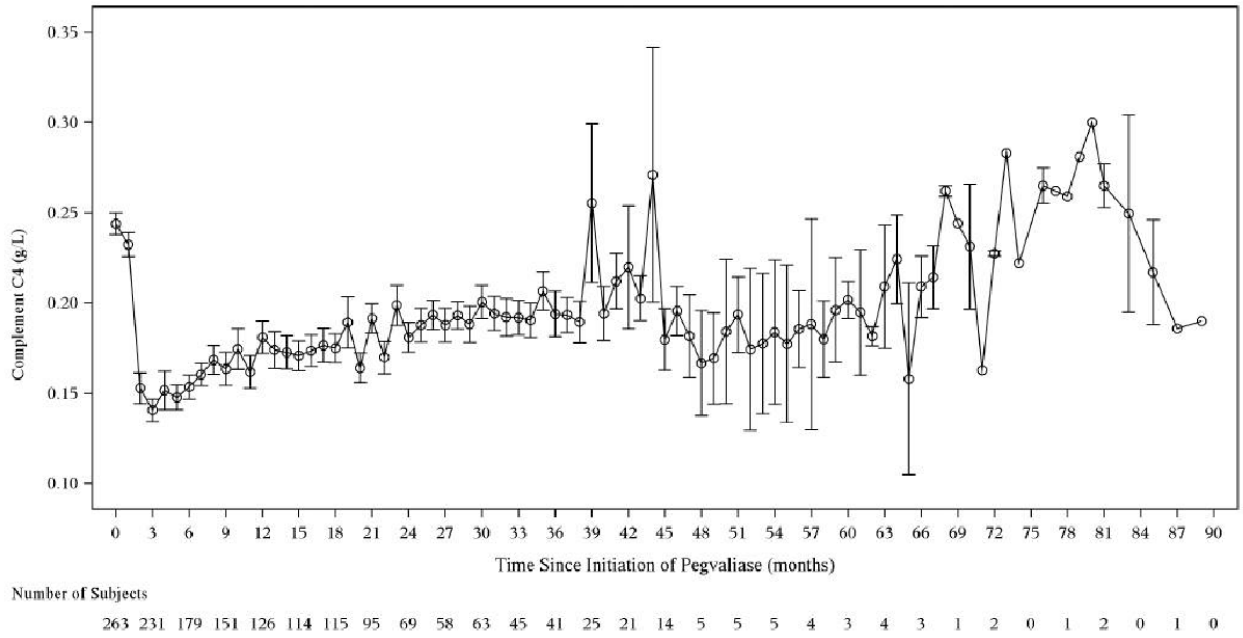
(Source of data: Figure 1.2 of Response to Clinical Pharmacology Information Request dated 19January2018; SDN 47)

Figure 22. Mean (SE) complement C3 over time for the Phase 3 Population



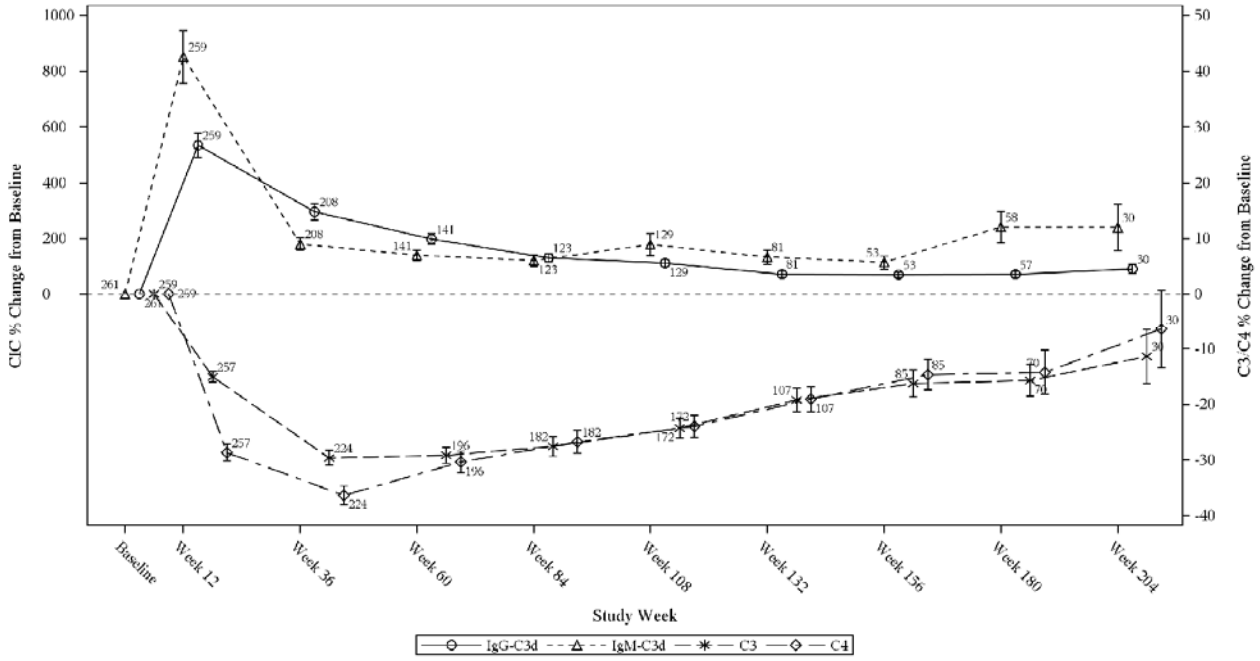
(Source of data: Figure 13.3.6.2 of Response to Clinical Pharmacology Information Request dated 19January2018; SDN 47)

Figure 23. Mean (SE) complement C4 over time for the Phase 3 Population



(Source of data: Figure 13.3.6.1 of Response to Clinical Pharmacology Information Request dated 19January2018; SDN 47)

Figure 24. Mean (SE) percent change in IgG-/IgM-C3d CIC and C3/C4 concentrations from baseline over time for Phase 3 Population with parent study 165-301

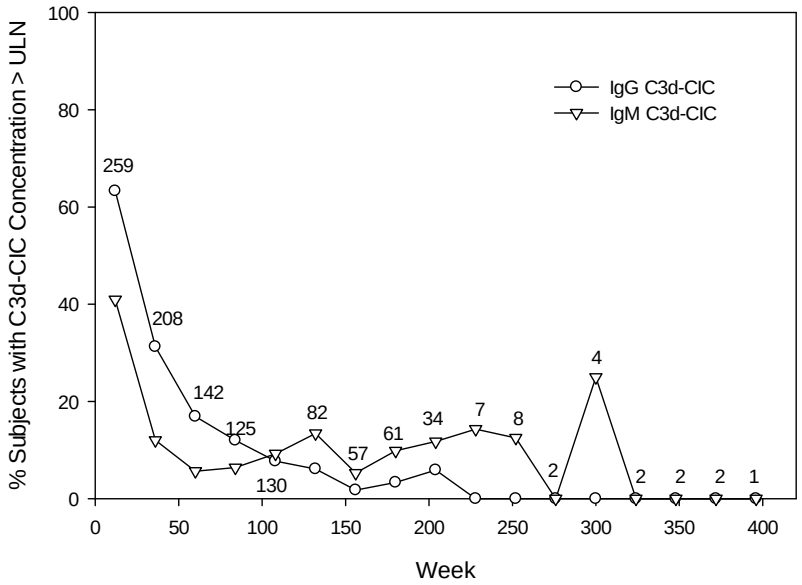


(Source of data: Figure 13.3.1.18 of Response to Clinical Pharmacology Information Request dated 19January2018; SDN 47)

The reviewer performed additional analyses to determine the incidence of CIC positivity over time. IgG C3d-CIC and IgM C3d-CIC concentrations were above the upper limit of normal (ULN) in approximately 60% and 40% of subjects, respectively, at Week 12 (**Figure 25**). The incidence of CIC positivity decreased over time, with IgG-C3d CIC concentrations > ULN (i.e., > 36 µgEq/mL) in 2 – 7% of subjects and IgM C3d-CIC concentrations > ULN (i.e., > 7.6 µgEq/mL) in 5 – 17% of subjects between two to four years (i.e., Week 108 to Week 204) after pegvaliase initiation.

Upon the Agency request, the Applicant performed and submitted similar analysis results for the incidence of C3 and C4 below the lower limit of normal (LLN; i.e., 0.9 g/L and 0.1 g/L, respectively). The incidence of concentrations below LLN peaked at Month 6 (~60%) and Month 3 (~40%) for C3 and C4, respectively. The incidence declined over time, with 43% and 14% of subjects had low C3 and C4 concentrations, respectively, at Month 45 (**Table 24**)

Figure 25. Incidence of CIC concentrations above upper limit of normal over time for the Phase 3 Population



(Source of data: Reviewer's analysis based on dataset "adcic.xpt" from 120-day Safety Update)

Table 24. Incidence of C3 and C4 concentrations below the lower limit of normal over time at selected timepoints for the Phase 3 Population

	Phase 3 Population (N = 273)	
Study Month	Subjects with C3 < LLN N (%)	Subjects with C3 < LLN N (%)
Baseline	19/263 (7.2%)	2/263 (0.8%)
M3	117/230 (50.9%)	92/231 (39.8%)
M6	110/179 (61.5%)	53/179 (29.6%)
M12	68/126 (54.0%)	25/126 (19.8%)
M18	58/115 (50.4%)	24/115 (20.9%)
M24	35/69 (50.7%)	6/69 (8.7%)
M36	18/41 (43.9%)	6/41 (14.6%)
M42	8/21 (38.1%)	0/21 (0.0%)
M45*	6/14 (42.9%)	2/14 (14.3%)

*The number of subjects with C3 and C4 assessment was small after Month 45 and not included in the table

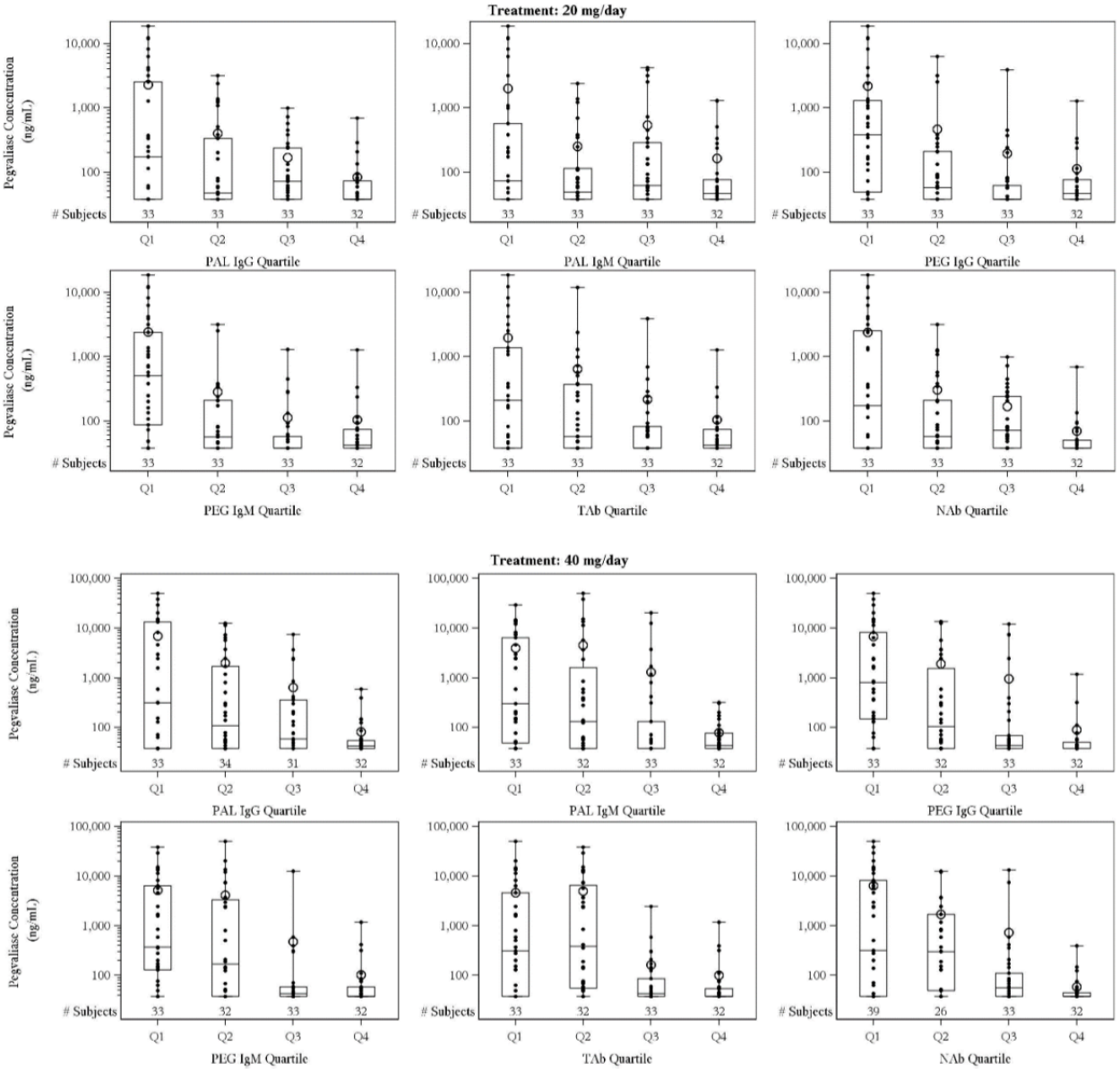
(Source of data: Adopted from Tables 13.3.6.18 and 13.3.6.14 of Response to Clinical Pharmacology Information Request dated 19January2018; SDN 47)

1.4.7 Impact of Immunogenicity on PK

Overall, an inverse relationship between ADA response and pegvaliase plasma exposure was observed in all clinical studies, suggesting that subjects who mount a stronger immune response to pegvaliase have lower plasma exposure.

In 165-301, the Applicant assessed the impact of immunogenicity on PK by plotting mean trough pegvaliase concentrations across the study for individual subjects by antibody quartiles that were based on the mean antibody titer across the study for individual subjects. There appeared to be an inverse relationship between antibody titers and pegvaliase trough concentrations for all anti-drug antibodies including anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, TAb, and NAb (**Figure 26**).

Figure 26. Mean trough pegvaliase concentrations by antibody titer quartiles and randomized dose group in 165-301



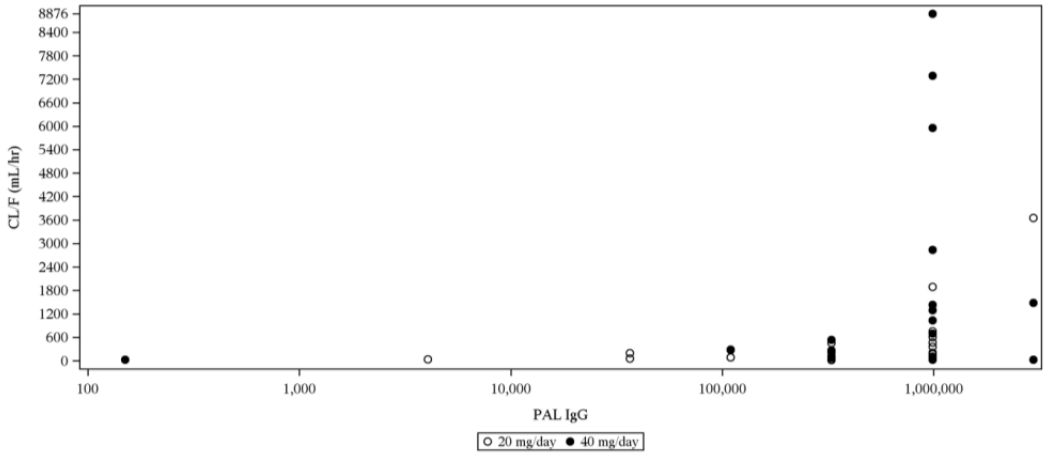
Note: Concentrations below limit of quantitation were replaced with half of the lower limit of quantitation (i.e. LLOQ/2 = 37.5 ng/mL) values in calculating the mean concentration of individual subjects. Horizontal lines from

bottom to top: minimum, first quartile, median, third quartile, and maximum values for individual mean trough pegvaliase concentration measurements. Circle: mean value for individual mean trough pegvaliase concentration measurements.

Of note, the Applicant used all pegvaliase concentrations collected pre-dose or post-dose in the above analysis, including 177 out of 1689 (10.9%) samples collected post-dose within 12 hours of dosing. All these pegvaliase concentrations were treated as trough concentrations without taking into account dosing or sampling deviations. Based on an estimated terminal half-life of 34 hours in the maintenance phase (from the population PK analysis results), the Applicant's estimated that the ratio of trough to peak concentration at steady state following daily administration to be 0.61, indicating that the magnitude of fluctuation in plasma pegvaliase concentration within the dosing interval is relatively small compared to the high inter-subject variability of pegvaliase PK.
(Source of data: Figure 4.2.4.2 of IRR)

In 165-302 Part 3, apparent clearance of pegvaliase (PFS) at steady state (CL_{ss}/F) assessed at Week 5/6 were plotted against the antibody titers measured at Week 4, a week prior to the PK assessment in Part 3. CL_{ss}/F appeared to be higher in subjects with higher titers for anti-PAL IgG (**Figure 27**). Similar trends were found for anti-drug antibodies including anti-PAL IgM, anti-PEG IgM, TAb, and NAb (see plots in Figure 14.2.7.15 of 165-302 CSR). The relationship between the CL_{ss}/F and anti-PEG IgG titer could not be assessed as there was no anti-PEG IgG detected.

Figure 27. Pegvaliase apparent clearance (CL_{ss}/F) of PFS and anti-PAL IgG titers in 165-302 Part 3



(Source of data: Figure 14.2.7.15 of 165-302 CSR)

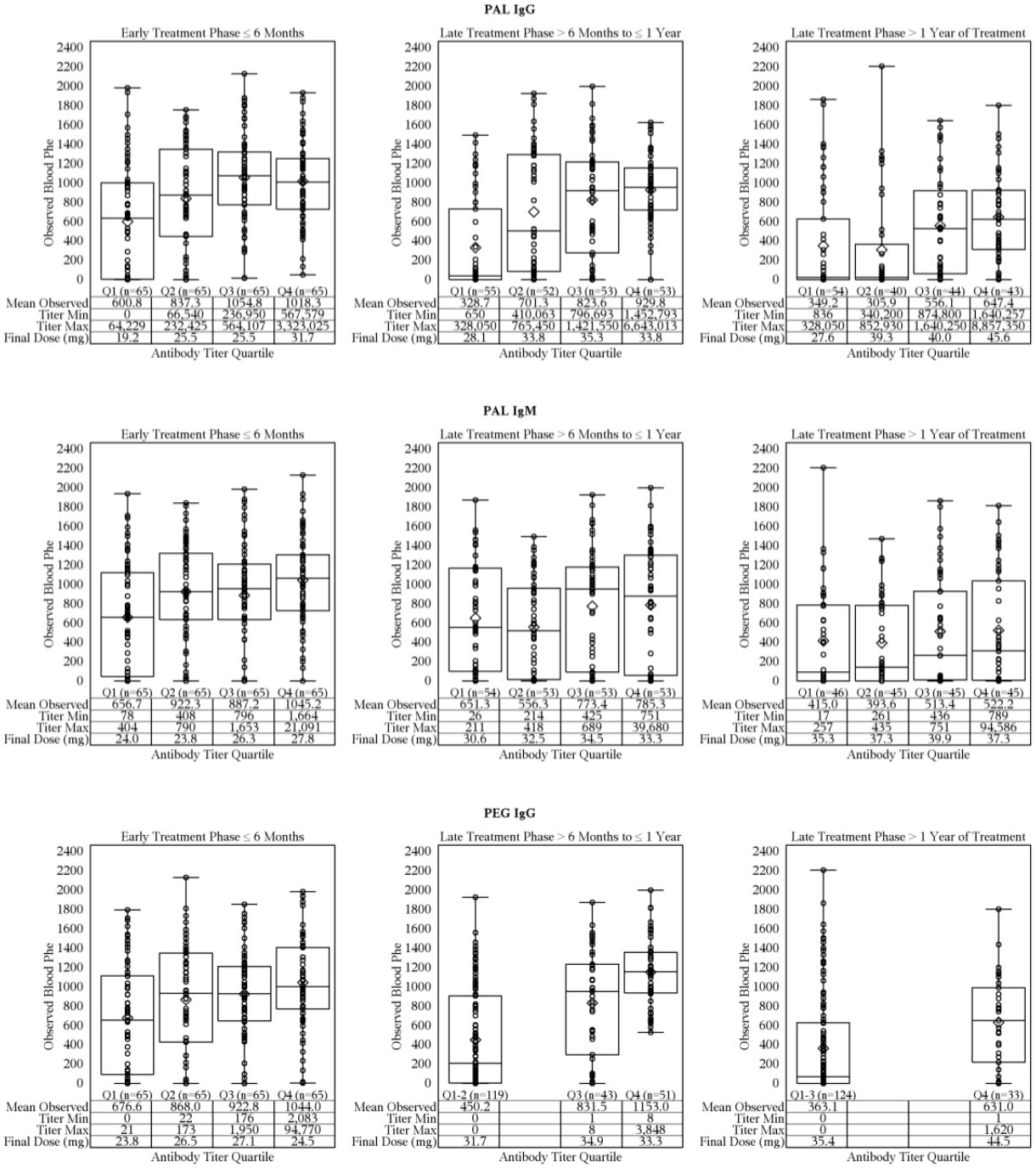
1.4.8 Impact of Immunogenicity on PD/Efficacy

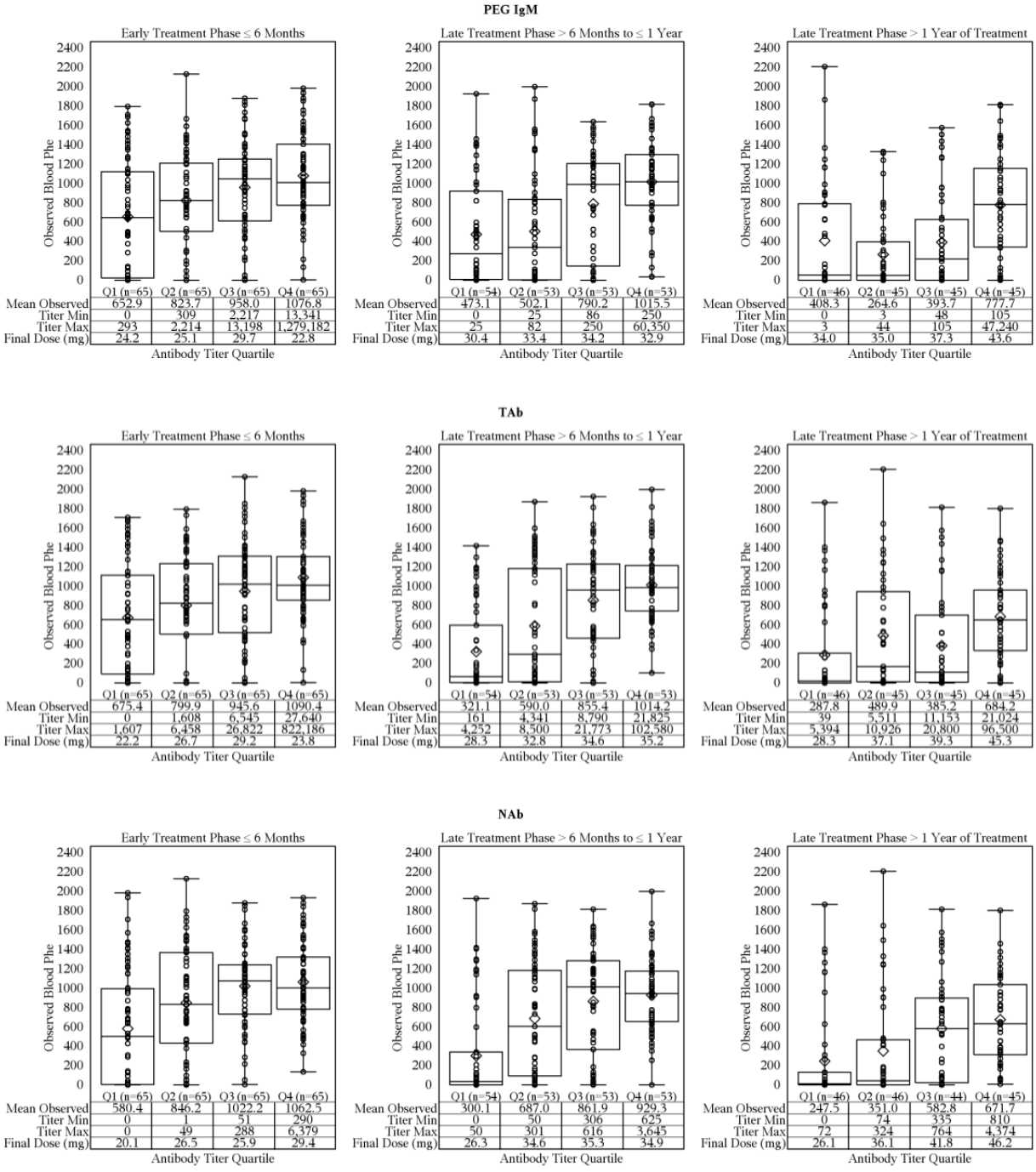
Overall, an inverse relationship between antibody responses and efficacy (i.e., blood Phe reduction) was observed in all studies; subjects with higher mean antibody titers experienced less blood Phe reductions. The impact of antibody response on blood Phe reduction is likely due to immune-mediated clearance of the drug from the plasma.

In an integrated analysis of Phase 3 studies (165-301 and 165-302), the Applicant evaluated the relationship between antibody titers and blood Phe reduction by plotting observed blood Phe concentration at the last available visit against antibody titer quartiles based on subjects' mean

antibody in early treatment (≤ 6 months) vs late treatment (> 6 months to 1 year and > 1 year). Overall, subjects in the higher antibody titer quartiles (i.e., Q3 and/or Q4), regardless of antibody analyte, experienced smaller reductions in blood Phe concentration (**Figure 28**).

Figure 28. Observed blood Phe concentration (umol/L) and antibody titer by treatment phase in Study 165-301





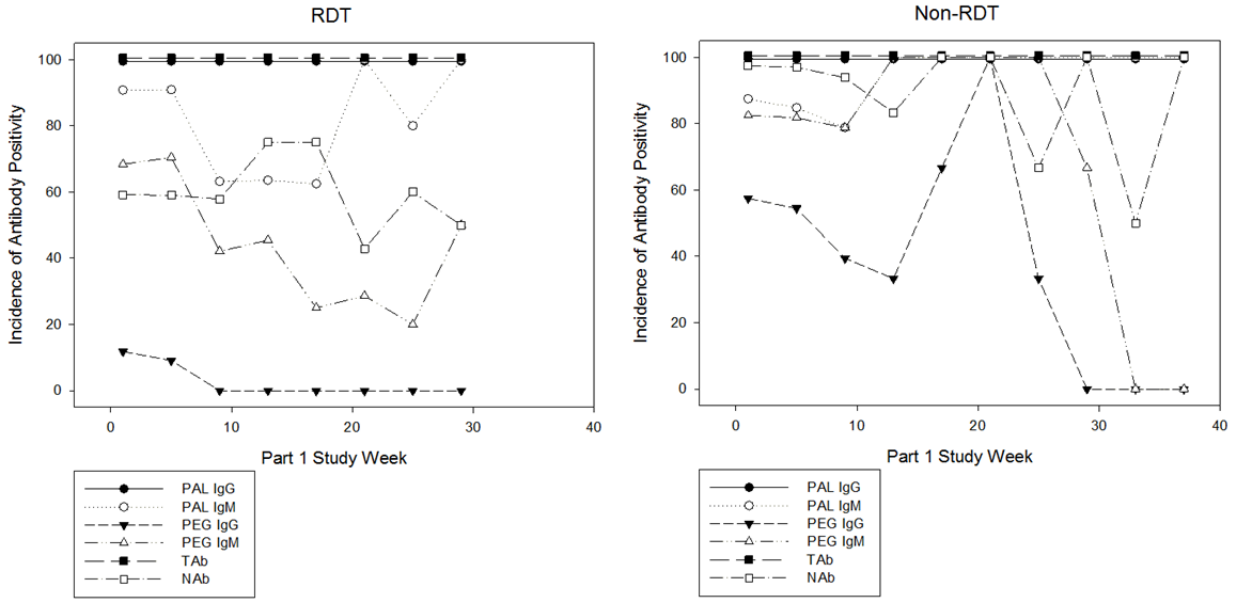
(Source of data: Figure 2.7.4.9.1.2 of 165-301 CSR)

In 165-302 Part 1, the Applicant compared the immunogenicity profile between the Randomized Discontinuation Trial (RDT) Population and the Non-RDT Population. The RDT Population represents subjects who reached the randomized dose of 20 mg/day or 40 mg/day and had a mean blood Phe reduction of ≥ 20% (using the last two consecutive blood Phe assessments of Part 1) from the baseline of 165-301 and entered Part 4. The Non-RDT

Population represents subjects who were originally enrolled in 165-301, did not participate in Part 2, and did not have ≥20% blood Phe reduction upon entry into Part 4.

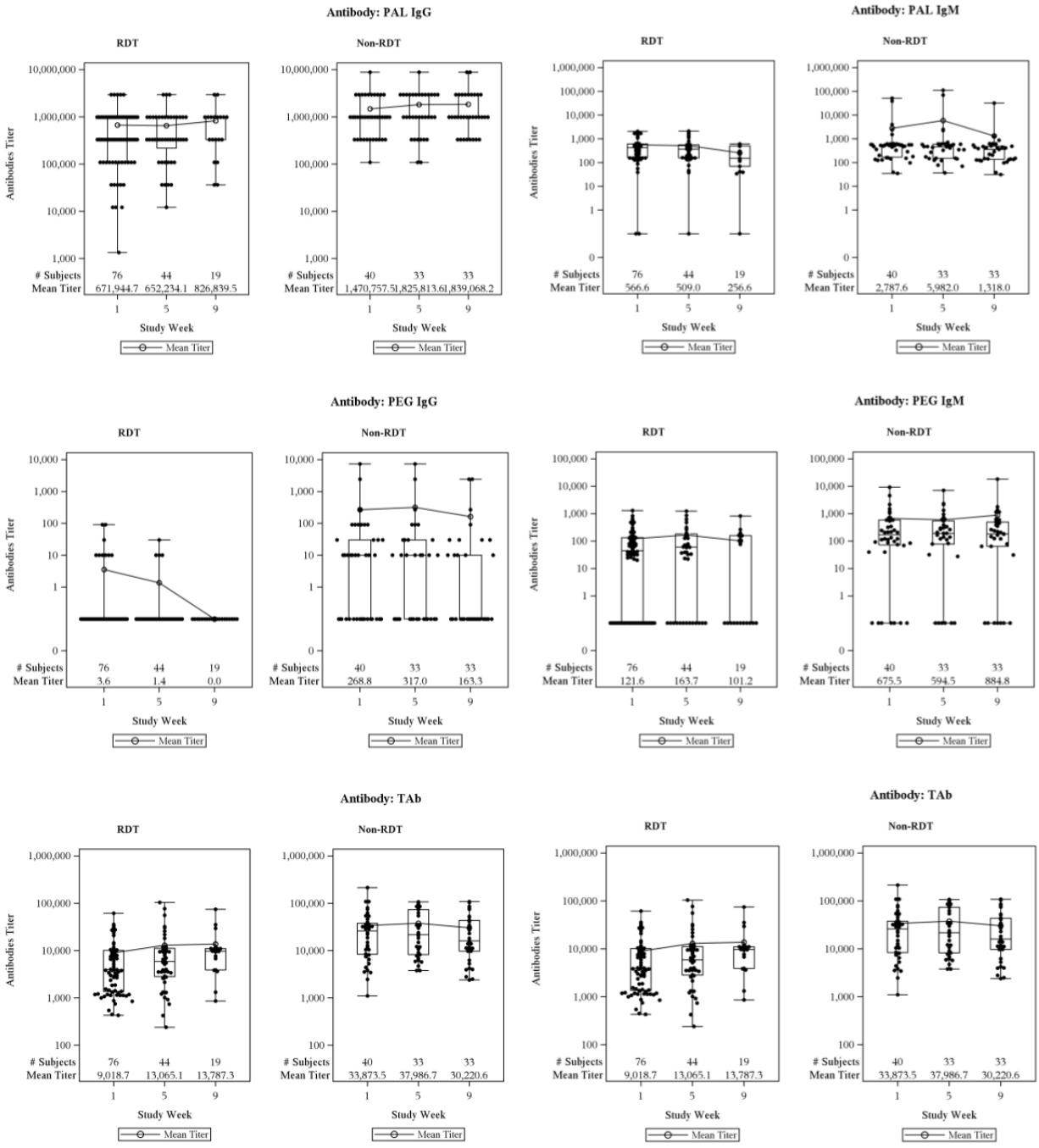
As depicted in **Figure 29**, more subjects were tested positive for anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, and NAb in the Non-RDT Population compared with subjects in the RDT Population, with the exception that all subjects in both populations were tested positive for anti-PAL IgG and TAb at all study visits. In addition, mean antibody titers were higher in the Non-RDT than in the RDT Population for all antibodies (**Table 30**). These results indicate that higher immune responses were associated with less reductions in blood Phe concentration to reach the target reduction of 20%.

Figure 29. Incidence of antibody positivity between RDT and Non-RDT in Study 165-302 Part 1



(Source of data: Reviewer's analysis based on data from Table 14.3.6.69 of 165-302 CSR)

Figure 30. Boxplot of antibody titers between RDT and Non-RDT in Study 165-302 Part 1



(Source of data: Figure 14.3.6.56 of 165-302 CSR)

In the Response to Request for Information dated November 3, 2017 from the Agency (SDN 25), the Applicant provided immunogenicity profile for three active subgroups of modified intention-to-treat (mITT) Population based on blood Phe concentration achieved and maintained throughout 165-302 Part 2.

There were no differences in antibody titers between the different blood Phe subgroups at pretreatment baseline. During 165-301, 165-302 Part 1, and at the beginning of 165-302 Part 2, mean ADA titers were generally lower in the < 120 µmol/L subgroup compared with the > 360 µmol/L subgroup, for most antibody analytes including anti-PAL IgG (**Table 25**), anti-PEG IgM, and NAb. The number of subjects in the 120 – 360 µmol/L subgroup was too small for comparison.

There were no differences in IgM CIC, IgG CIC, C3 and C4 concentrations between the different blood Phe subgroups at pretreatment baseline. During 165-301, IgM CIC and IgG CIC concentrations were slightly lower for the <120 µmol/L subgroup compared with the > 360 µmol/L subgroup but with overlapping standard deviations. No differences were observed in C3 or C4 concentrations between the <120 µmol/L subgroup or > 360 µmol/L subgroup at any of the evaluated time points.

These results are consistent with the negative impact of antibody titers on blood Phe reduction as described above in this section i.e., subjects with higher antibody titers experienced smaller reductions in blood Phe levels. See Tables 9.2.3 and 9.2.4 for all immunogenicity results in the Response to Request for Information dated November 3, 2017.

Table 25. Summary of immunogenicity profile for active subgroups of mITT Population

	Blood Phe Level Subgroups for Active Subjects in mITT Population (n=48)			All Active Subjects (n=58)	Placebo (n=28)	mITT Population (N=86)
	<120 µmol/L (n=21)	120 to 360 µmol/L (n=2)	>360 µmol/L (n=25)			
PAL IgG						
Parent study baseline						
n	20	2	25	57	27	84
Mean (SD)	97.5 (401.5)	0.0 (0.0)	18.0 (90.0)	43.9 (244.8)	3.7 (13.3)	31.0 (202.1)
Median	0.0	0.0	0.0	0.0	0.0	0.0
Parent Study Entire Duration						
n	17	2	24	50	27	77
Mean (SD)	184,090.2 (196,383.1)	441,115.6 (99,750.7)	544,683.9 (781,815.1)	368,144.1 (580,282.5)	435,096.1 (497,499.4)	391,620.8 (550,279.8)
Median	97,216.7	441,115.6	201,227.8	146,277.8	251,650.0	220,387.5
165-302 Part 1 Entire Duration						
n	21	2	25	58	28	86
Mean (SD)	330,428.6 (290,956.4)	984,150.0 (0.0)	1,100,304.0(1,237,144.5)	698,588.4 (910,197.3)	690,986.5 (513,586.1)	696,113.4 (799,596.0)
Median	328,050.0	984,150.0	656,100.0	328,050.0	656,100.0	328,050.0
Part 2 baseline 165-302						
n	21	2	25	58	28	86
Mean (SD)	412,007.1 (346,565.7)	984,150.0 (0.0)	952,074.0 (955,398.0)	657,589.7 (723,136.1)	987,235.7 (1,009,691.4)	764,916.3 (835,848.8)
Median	328,050.0	984,150.0	984,150.0	328,050.0	984,150.0	328,050.0

	Blood Phe Level Subgroups for Active Subjects in mITT Population (n=48)			All Active Subjects (n=58)	Placebo (n=28)	mITT Population (N=86)
	<120 µmol/L (n=21)	120 to 360 µmol/L (n=2)	>360 µmol/L (n=25)			
IgG-C3d CIC						
Parent Study Baseline						
n	17	2	24	50	26	76
Mean (SD)	11.8 (7.5)	10.3 (5.4)	10.4 (4.6)	10.8 (5.6)	10.1 (5.8)	10.6 (5.6)
Median	11.5	10.3	9.1	9.4	9.2	9.2
Parent Study Entire Duration						
n	17	2	24	50	27	77
Mean (SD)	29.4 (11.9)	43.3 (25.4)	41.2 (20.7)	38.9 (25.9)	33.1 (21.6)	36.9 (24.5)
Median	26.6	43.3	35.5	31.3	28.3	30.4
165-302 Part 1 Entire Duration						
n	NA	NA	3	4	1	5
Mean (SD)			20.6 (12.3)	20.6 (10.1)	26.2 (NA)	21.7 (9.1)
Median			15.7	18.1	26.2	20.5
Part 2 baseline (165-302)						
n	NA	NA	3	3	1	4
Mean (SD)			41.5 (4.3)	41.5 (4.3)	25.8 (NA)	37.6 (8.6)
Median			40.7	40.7	25.8	39.2
C3						
Parent Study Baseline						
n	17	2	24	50	25	75
Mean (SD)	1.1 (0.2)	1.0 (0.1)	1.1 (0.2)	1.1 (0.2)	1.2 (0.3)	1.2 (0.2)

	Blood Phe Level Subgroups for Active Subjects in mITT Population (n=48)			All Active Subjects (n=58)	Placebo (n=28)	mITT Population (N=86)
	<120 µmol/L (n=21)	120 to 360 µmol/L (n=2)	>360 µmol/L (n=25)			
Median	1.1	1.0	1.1	1.1	1.2	1.2
Parent Study Entire Duration						
n	17	2	24	50	27	77
Mean (SD)	0.9 (0.3)	0.7 (0.1)	0.8 (0.2)	0.8 (0.2)	1.0 (0.3)	0.9 (0.3)
Median	0.9	0.7	0.8	0.8	1.0	0.9
165-302 Part 1 Entire Duration						
n	12	2	13	32	15	47
Mean (SD)	0.8 (0.2)	0.5 (0.2)	0.8 (0.2)	0.8 (0.2)	0.9 (0.4)	0.8 (0.3)
Median	0.9	0.5	0.9	0.8	0.8	0.8
Part 2 baseline (165-302)						
n	21	2	25	58	28	86
Mean (SD)	0.9 (0.2)	0.5 (0.2)	0.8 (0.2)	0.8 (0.3)	0.9 (0.3)	0.8 (0.3)
Median	0.9	0.5	0.8	0.8	0.8	0.8

C3, complement 3; CIC, circulating immune complex; Ig, immunoglobulin; Max, maximum; Min, minimum; mITT, modified Intent-to-Treat (Population); NA, not applicable; NAb, neutralizing antibodies; SD, standard deviation; TAb, total antibodies.

The mITT Population included all subjects who reached the randomized dose of 20 mg/day or 40 mg/day and were randomized into Part 2 with a mean blood Phe reduction of ≥ 20% (using the last two consecutive blood Phe assessments of Part 1) from the baseline of 165-301 or the Phase 2 study in which they initiated pegvaliase. Active subjects in the mITT Population of Part 2 of 165-302 were categorized into 3 subgroups based on blood Phe levels during Part 2 of 165-302. Subjects had to have all blood Phe levels within the subgroup level to have been included.

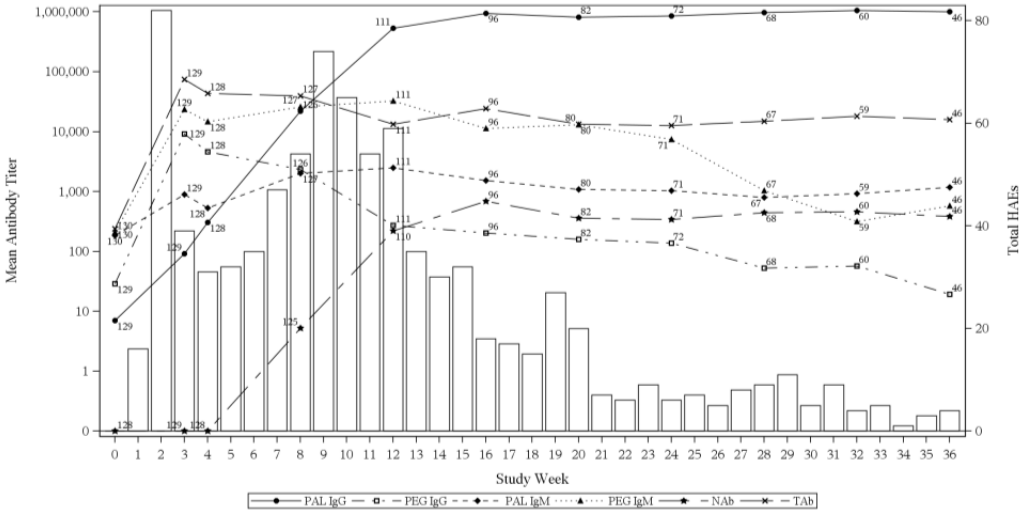
(Source of data: Table 3b.1 of Response to Request for Information dated November 3, 2017; SDN 25)

1.4.9 Impact of Immunogenicity on Safety

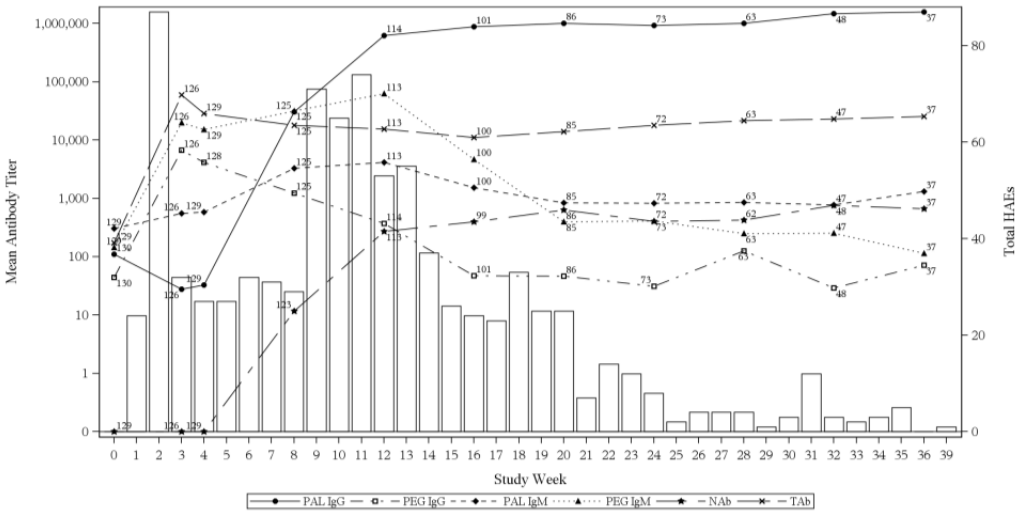
Overall, data demonstrated an association between antibody response and adverse events (AEs) in pegvaliase clinical studies. Typically, hypersensitivity reactions (HAEs) occurred most frequently during the first six months of treatment when the early immune response comprised of anti-PEG IgM, anti-PEG IgG, and anti-PAL IgM responses peaked (**Figure 31**), C3/C4 concentrations declined (**Figure 22** and **Figure 23**), and CIC concentrations were at their highest (**Figure 21**). The frequency of HAEs decreased over time in long term treatment as the incidence of these antibodies decreased, and C3/C4 and CIC levels returned towards baseline (**Figure 31**).

Figure 31. Frequency of HAEs and antibody titer over time for the (a) 20 mg/day and (b) 40 mg/day group

(a) 20 mg/day



(b) 40 mg/day



(Source of data: Figure 14.3.6.3.5 of Study 165-301 CSR)

In general, an association between antibody titers and frequency of HAEs was observed for some antibody analytes, such that subjects who had lower mean titers experienced fewer HAEs. As depicted in **Table 26**, more HAEs occurred in subjects with antibody titers above the median values (Q3+Q4) than subjects with antibody titers below the median values (Q1+Q2) for anti-PAL IgM, TAb, and NAb after one year of study treatment. No such association was observed for anti-PAL IgG, anti-PEG IgG, or anti-PEG IgM, but the study results for anti-PEG IgG ADAs should be interpreted with caution. See Section 1.1.3.1 for more information.

Likewise, mean anti-PAL IgG and TAb titers tended to be higher in subjects who experienced AEs of significance (e.g., generalized skin reactions ≥ 14 days, injection site reactions ≥ 14 days, injection site reactions, and arthralgia) in 165-302 Parts 1 and 4 **(Table 27**).

Table 26. Frequency of HAEs and antibody titer quartiles by treatment phase for Phase 3 Population initially enrolled in Study 165-301

	Mean number of HAEs					
	≤ 6 Months		> 6 Months to ≤ 1 Year		> 1 Year	
	Q1 + Q2	Q3 + Q4	Q1 + Q2	Q3 + Q4	Q1 + Q2	Q3 + Q4
Anti-PAL IgG	7.0	8.35	2.5	3.95	7.1	5.85
Anti-PAL IgM	8.0	7.45	3.0	3.65	3.65	8.85
Anti-PEG IgG	7.25	8.25	4	2.6	7.2	5.2
Anti-PEG IgM	7.7	7.8	3.65	2.9	6.1	6.6
TAb	7.05	8.35	3.3	3.3	3.65	9.15
NAb	7.4	8.0	3.35	3.4	4.35	8.9

(Source of data: Reviewer's analysis based on data from Figure 2.7.4.9.1.1 of ISS)

Table 27. Mean antibody titers and AEs of significance in Study 165-302 Part 1 and Part 4

AE of Interest	Mean Titer					
	Anti-PAL IgG	Anti-PAL IgM	Anti-PEG IgG	Anti-PEG IgM	TAb	NAb
Part 1						
GSSs ≥ 14 days						
Yes (N = 7)	1,248,413	330	3.3	86.4	28,488	457
No (N = 157)	953,007	1,461	70.8	330	18,162	527
ISRs ≥ 14 days						
Yes (N = 13)	1,778,440	1,480	17.0	590	34,372	711
No (N = 151)	895,638	1,407	72.4	297	17,245	508
ISRs						
Yes (N = 54)	1,443,834	1,473	189	596	27,124	777
No (N = 110)	730,855	1,383	8.7	185	14,420	400
Arthralgia						
Yes (N = 22)	1,361,076	611.3	8.1	285.5	26,094	4567
No (N = 142)	904,348	1,537	77.2	325.3	17,442	535
Part 4						
GSSs ≥ 14 days						
Yes (N = 53)	1311792	1217	19	2732	17762	659
No (N = 149)	1103609	2061	27	596	14531	476
ISRs ≥ 14 days						
Yes (N = 56)	1174880	2471	14	1737	16960	527
No (N = 146)	1151845	1597	29	933	14773	523
ISRs						
Yes (N = 127)	1295491	1543	33	1021	17667	595
No (N = 75)	925804	2342	12	1385	11505	404
Arthralgia						
Yes (N = 73)	1186747	2663	15	2295	15729	577
No (N = 129)	1142094	1374	31	512	15181	494

GSS; generalized skin reactions; ISRs: injection site reactions

(Source of data: Tables 14.3.6.81 to 14.3.6.84 and Tables 14.3.6.105 to 14.3.6.108 of 165-302 CSR)

Overall, more subjects in the higher IgG-C3d CIC quartiles (i.e., Q3 and Q4; 41.1%) discontinued from pegvaliase study than the subjects in the lower quartiles (i.e., Q1 and Q2; 16.9%) (**Table 28**). In addition, higher percentage of subjects in Q3 and Q4 (i.e., 14.7%) discontinued pegvaliase study due to adverse events compared to percentage of subjects in Q1 and Q2 (i.e., 2.3%). The incidences of serious adverse events (SAE) and anaphylaxis were higher in subjects with the greatest IgG-C3d CIC change (i.e., Q4) than the remaining subjects.

Table 28. Primary reason for study discontinuation and AEs by IgG-C3d CIC change from baseline quartiles for the Phase 3 Population initially enrolled in 165-301

	Q1 (N = 65)	Q2 (N = 65)	Q3 (N = 65)	Q4 (N = 64)	Total (N = 259)
Subjects discontinued from study	**9 (13.8%)**	**13 (20.0%)**	**25 (38.5%)**	**28 (43.8%)**	75 (29.0%)
Primary reason for study D/C					
AE	**1 (1.5%)**	**2 (3.1%)**	**7 (10.8%)**	**12 (18.8%)**	22 (8.5%)
Withdrawal by subject	1 (1.5%)	7 (10.8%)	10 (15.4%)	11 (17.2%)	29 (11.2%)
Lost to follow-up	3 (4.6%)	1 (1.5%)	4 (6.2%)	0	8 (3.1%)
Physician decision	2 (3.1%)	1 (1.5%)	2 (3.1%)	3 (4.7%)	8 (3.1%)
Other	2 (3.1%)	2 (3.1%)	2 (3.1%)	2 (3.2%)	8 (3.1%)
Quartile range	(-4.02, 0.79)	(0.79, 1.98)	(2.01, 5.53)	(5.59, 50.96)	
Any AE	65 (100%)	65 (100%)	65 (100%)	64 (100%)	259 (100%)
Any SAE	**12 (18.5%)**	**10 (15.4%)**	**9 (13.8%)**	**15 (23.4%)**	46 (17.8%)
Any HAE	58 (89.2%)	61 (93.8%)	62 (95.4%)	61 (95.3%)	242 (93.4%)
Any anaphylaxis per NIAID/FAAN criteria	**3 (4.6%)**	**7 (10.8%)**	**5 (7.7%)**	**12 (18.8%)**	27 (10.4%)
Arthralgia/arthrithis	39 (60.0%)	49 (75.4%)	46 (70.8%)	51 (79.7%)	185 (71.4%)
GSS ≥ 14 days	28 (43.1%)	26 (40.0%)	24 (36.9%)	19 (29.7%)	97 (37.5%)
Persistent urticaria ≥ 14 days	3 (4.6%)	4 (6.2%)	1 (1.5%)	2 (3.1%)	10 (3.9%)
Lymphadenopahty ≥ 14 days	7 (10.8%)	6 (9.2%)	9 (13.8%)	9 (14.1%)	31 (12.0%)
Modified serum sickness-like reaction	1 (1.5%)	1 (1.5%)	1 (1.5%)	4 (6.3%)	7 (2.7%)
Infection and infestations SOC ≥ 30 days	18 (27.7%)	19 (29.2%)	14 (21.5%)	8 (12.5%)	59 (22.8%)
Infection SAE	2 (3.1%)	1 (1.5%)	2 (3.1%)	1 (1.6%)	6 (2.3%)

D/C, discontinuation; AE, adverse event; SAE, serious adverse event; HAE, hypersensitivity adverse event; GSS, generalized skin reactions

(Source of data: Tables 2.7.4.9.22.2 and 2.7.4.9.9.2 of ISS)

1.4.10 Anti-Pegvaliase IgE Antibodies and Anaphylaxis

For the I/T/M population, 37 cases of anaphylaxis were identified in 26 subjects (see Dr. Stacy Chin's review dated March 1, 2018 for more information). Twenty-five of the 26 subjects were tested for the anti-pegvaliase IgE antibodies using the ImmunoCAP assay (validation report 21120-4767) and one subject did not give consent for IgE testing. Of the 25 subjects tested, 24 subjects were tested negative for anti-pegvaliase IgE antibodies. One subject screened positive in the ImmunoCAP anti-pegvaliase IgE assay but had insufficient sample for the confirmation step to confirm IgE positivity.

The serum samples analyzed for anti-pegvaliase IgE were collected at different timepoints around the anaphylaxis reactions, ranging from 42 days prior to and 51 days after the reactions. Pegvaliase concentrations were not available in four of these immunogenicity samples to assess the impact of drug tolerance. PK concentrations were below the drug tolerance of the anti-pegvaliase IgE assay (i.e., 25 µg/mL) and would not interfere the assay for the rest of the

immunogenicity samples; these PK concentrations were obtained on the same day or within 36 days of the immunogenicity samples.

Anti-PAL and anti-pegvaliase IgE antibodies were assessed during routine study visits (not at times of anaphylaxis episodes) in all Phase 2 subjects and during hypersensitivity visits only in 44 subjects initially enrolled in Study 165-301 (according to Response to FDA Labeling Comments Received 11May2018; SDN 76). Anti-PAL IgE antibodies were detected at least once in 11 of 124 (8.9%) subjects tested, but none of the samples were tested positive for anti-pegvaliase IgE antibodies (**Table 29**).

Table 29. Summary of anti-PAL and anti-pegvaliase IgE antibodies in Phase 2 and 3 studies

Parent Study (Includes data from all subsequent studies)	Ever Positive on Treatment	
	Anti-PAL IgE	Anti-Pegvaliase IgE
PAL-002 (N = 40)	5/40 (12.5%)	0/40
PAL-004 (N = 16)	1/16 (6.3%)	0/16
165-205 (N = 24)	4/24 (16.7%)	0/24
165-301 (N = 261)	1/44 (2.3%)	0/44
All subjects (N = 341)	11/124 (8.9%)	0/124

(Source of data: Table 2.7.4.8.1.7 of 120-Day Safety Report Part 2)

Reviewer's Comments

While Table 2.7.4.8.1.7 in the 120 Day Safety Update Report indicates that no subjects were tested positive for anti-pegvaliase IgE antibodies, PAL-002 CSR describes one subject having positive results for anti-pegvaliase IgE antibodies on Days 99 and 113 (Listing 16.2.8.3.4 of PAL-002 CSR).

1.4.11 Antidrug Antibodies between 20 mg/day and 40 mg/day Maintenance Dose in 165-301

Overall, the incidences of antibody positivity were in similar ranges between the 20 mg/day and the 40 mg/day groups, with slightly different incidence for anti-PAL IgG and NAb at specific weeks. In the 20 mg/day group, 28.7% and 44.5% subjects were positive for anti-PAL IgG at Weeks 3 and 4, respectively. On the other hand, 16.7% and 25.6% subjects were positive for anti-PAL IgG at Weeks 3 and 4, respectively, in the 40 mg/day group. As for NAb, the incidence of positivity was approximately 10% higher in the 20 mg/day group compared with the 40 mg/day group from Weeks 24 to 36 (**Table 16**).

The titer values did not seem to be different between the 20 mg/day and the 40 mg/day groups for anti-PAL IgG, anti-PAL IgM, anti-PEG IgG, anti-PEG IgM, TAb, and NAb in 165-301 (**Table 17** to **Table 19**).

1.5 Biopharmaceutics

1.5.1 Drug substance (DS)

The production process of pegvaliase formulated bulk drug substance (FBDS) consists of (b) (4)

During clinical development, improvements were made (b) (4) (b) (4). The overall process (b) (4) is the Phase 3 and proposed commercial process for manufacturing pegvaliase. See Product Quality Review for detailed DS information.

1.5.2 Drug Product (DP)

The pegvaliase DP is formulated and supplied as a clear sterile solution (b) (4) for clinical use. See Product Quality Review for detailed DP information.

(b) (4) The vial and syringe (VS) configuration was used in the pegvaliase clinical trials (165-301 and 165-302 Parts 1, 2, and 3A).

The (b) (4) packaging configuration, which is intended for commercial use, is a single-use PFS with staked needle for SC injection. Each PFS is filled to deliver at least 0.5 or 1 mL of formulation containing 2.5, 10, or 20 mg of pegvaliase (expressed as the amount of rAvPAL conjugated to 7.25, 29, and 58 mg of 20 kD PEG). Pegvaliase concentration is 5 mg/mL for the 2.5 mg PFS and is 20 mg/mL for the10 mg and 20 mg PFS configurations (165-301 and 165-302 Parts 3B and 4).

The excipients of the drug product in the VS and the PFS remained the same throughout clinical development and are summarized in **Table 30**.

Table 30. Drug product composition provided in vial and in prefilled syringe

Components	(b) (4)	Prefilled Syringe		
		2.5 mg/syringe	10 mg/syringe	20 mg/syringe
Pegvaliase (rAvPAL-PEG)		2.5	10	20
Tromethamine		(b) (4)		
Tromethamine-HCl (Tris-HCl)				
NaCl				
Trans-cinnamic acid		0.07	0.07	0.15
Water for Injection (qs)		0.5 mL	0.5 mL	1.0 mL

(source: Tables 2.7.1.1.1 and 2.7.1.1.2 of Summary of Biopharmaceutics Studies and Associated Analytical Methods)

1.5.3 Drug Products Used in Clinical Trials

The VS and PFS drug products were both used in the Phase 3 trials. Specifically, the VS and PFS was used in 165-301. In 165-302, the VS drug product was used in Parts 1, 2, and 3A, whereas the PFS drug product was used in Parts 3B and 4.

Reviewer's comments*: The available PK data are not adequate to establish that VS and PFS products are comparable (see Section 1.2). The PK comparability data in Part 3 of study 165-302 showed that PFS had approximately 52% higher exposure compared to VS for the combined 20 mg and 40 mg doses. The exact reason for the observed PK differences is not clear.*

Note that the VS products (15 mg/1 mL, and 20 mg/1.3 mL) and PFS products (10 mg/0.5mL, and 20 mg/1 mL) have the same excipient formulation with a small difference in pegvaliase concentration (15 mg/mL versus 20 mg/mL). The presentation difference between liquid in vial and prefilled syringe in general would not result in PK difference following manual SC administration. Therefore, the formulation/presentation difference between VS and PFS could not explain the PK differences observed. The observed PK differences were attributable to several factors, including (1) large between-subject and within-subject PK variability under the influence of immunogenicity, (2) limited number of subjects, and (3) limitation of the study design where all subjects in the PK substudy received sequential administration of VS in Part 3A followed by PFS in Part 3B.

As both VS and PFS products have demonstrated clinical efficacy in reducing blood Phe concentrations across clinical trials and the Applicant has only proposed to market the PFS presentation for pegvaliase, the lack of PK comparability between VS and PFS presentation would not represent an approvability issue from a Clinical Pharmacology perspective. Furthermore, because the to-be-marketed PFS presentation was used in clinical trials, there is no need to bridge the to-be-marketed drug product to the clinical trial drug product. Because dosing of pegvaliase is individually titrated based on patient tolerability and blood Phe

concentration, the remaining PK comparability uncertainties (if there are any) between these product presentations would not affect the effectiveness of pegvaliase.

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/s/

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U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research
Office of Translational Sciences
Office of Biostatistics

STATISTICAL REVIEW AND EVALUATION

STATISTICAL CONSULTATION

BLA #:	761079
Drug Name:	PALYNZIQ® (pegvaliase)
Indication(s):	Reduce blood phenylalanine levels in adult patients with phenylketonuria
Applicant:	Biomarin Pharmaceutical Inc.
Date(s):	June 30, 2017 (Document date)
	November 19, 2017 (Consult date)
Safety endpoint	Anaphylaxis and hypersensitivity adverse events
Review Priority:	Standard
Biometrics Division:	Division of Biometrics VII
Statistical Reviewer:	Tae Hyun Jung, Ph.D.
Concurring Reviewers:	Clara Kim, Ph.D., Team Leader
	Mark Levenson, Ph.D., Division Director
Medical Division:	OND/ODEIII/DGIEP
Clinical Team:	Irena Lavine, M.D.
	Patroula Smpokou, M.D., Team Leader
Project Manager:	Benjamin Vali, M.S.

Keywords: phenylketonuria (PKU)**,** hypersensitivity adverse events, anaphylaxis, enzyme replacement therapy

1 INTRODUCTION

PALYNZIQ® (pegvaliase) is a PEGylated phenylalanine-metabolizing enzyme indicated to reduce blood phenylalanine (Phe) concentrations in adult patients with phenylketonuria (PKU) who have uncontrolled blood Phe concentrations greater than 600 µmol/L on existing management. The proposed dosing follows an induction, titration, and maintenance dosage regimen by starting at 2.5 mg once weekly and titrate up to 20 mg once daily or 40 mg once daily. To reach full effect, a patient should stay at 20 mg daily for 24 to 32 weeks, after which the dose may be increased to 40 mg daily based on individual patient response (Phe concentration) and tolerability. The formulation of pegvaliase is clear sterile solution. BioMarin Pharmaceutical Inc. submitted an original BLA for the approval of pegvaliase on June 30, 2017.

The sponsor identified a high rate of hypersensitivity adverse events including anaphylaxis; and initiated risk mitigation strategies mid trial in May 2014. The risk mitigation strategies were requiring (1) mandatory premedication, (2) injectable epinephrine, (3) training and education on how to recognize and respond to hypersensitivity reactions including anaphylaxis, and (4) the presence of a responsible adult during and for one hour after dosing for the first 16 weeks.

One of the two pivotal studies included treatment-naïve patients only (trial 165-301), who after completion were eligible to enroll in the other pivotal trial (165-302). Trial 165-301 consisted of induction, titration, and maintenance phases. The titration phase duration was individualized, up to 30 weeks, according to the time needed to titrate the dose up to the target dose.

On November 19, the Division of Gastroenterology and Inborn Errors Products (DGIEP) consulted the Division of Biometrics VII (DB7) to address the following safety issues that stemmed from the complicated study designs of the pivotal trials.

1. Appropriateness of using "events/person-years" to adjust for varying durations of exposure in patients.
2. Alternative approaches to comparing event incidences in trial 165-301, especially during the titration period when the dose was not stable.
3. Best ways to integrate safety analyses (e.g., pooling) in the context of treatment-naïve versus non-treatment naïve patients.
4. Best ways to determine the impact of risk mitigation strategies before and after May 2014.

This review includes the summary of the pivotal trials, and DB7's responses to the consult questions.

2 DATA SOURCES

Data used in this review are located at \\CDSESUB1\evsprod\BLA761079\0001\m5\datasets\165-301\analysis\adam\datasets.

The sponsor additionally submitted adaea.xpt in response to our information request, dated February 16, 2018, located at \\CDSESUB1\evsprod\BLA761079\0051\m5\datasets\iss-120-day\analysis\adam\datasets.

3 STUDY DESIGNS

3.1 Study 165-301

Study 165-301 is a Phase 3, open-label, multicenter study to evaluate the safety and tolerability during induction, titration, and maintenance dose regimens in pegvaliase-naïve subjects who self-administered pegvaliase up to fixed maintenance dose levels of 20 mg/day and 40 mg/day. PKU patients ≥18 years old with a blood Phe concentration of >600 µmol/L and no previous exposure to pegvaliase were eligible to participate. A total of 261 subjects were randomized (1:1) to titrate up to maintenance doses of either 20 mg/day or 40 mg/day pegvaliase administered subcutaneously. Dosing continued up to 36 weeks in 3 sequential periods:

- *Induction Period (Week 1 to Week 4)*: All subjects initiated pegvaliase at a fixed-dose induction of 2.5 mg/week for 4 weeks.
- *Titration Period (Week 5 to up to Week 34)*: Following completion of the Induction Period, the pegvaliase dose was titrated upwards from a weekly dose regimen to a daily dose regimen of 20 mg/day or 40 mg/day for up to 30 additional weeks. Subjects completed titration once they achieved the randomized dose regimen of 20 mg/day or 40 mg/day. Subjects who did not reach the randomized dose level by Week 34 due to adverse events (AEs) maintained the tolerated dose for the last 2 weeks of the study.
- *Maintenance Period (at least 3 weeks in duration)*: After reaching their randomized 20 or 40 mg/day dose level for 1 week of dosing, subjects maintained that dose level for at least 2 additional weeks until they reached a minimum of approximately 26 weeks or a maximum of 36 weeks in the study. At the discretion of the investigator and medical monitor, subjects who experienced a rapid decrease to low blood Phe levels and maintained their randomized dose level for 3 weeks were eligible to transition to Study 165-302 prior to completing the minimum 26 weeks. In addition, because the sponsor closed the study, 51 subjects transitioned to Study 165-302 prior to Week 26; these subjects were considered study completers.

Study 165-301 was designed to provide a pool of subjects eligible to enroll into Study 165-302, the pivotal placebo-controlled randomized discontinuation trial.

3.2 Study 165-302

Study 165-302 is a four-part, Phase 3, randomized, double-blind, placebo-controlled, four-arm, discontinuation study to evaluate the efficacy and safety of subcutaneous injection of pegvaliase self-administered by adults with PKU. Only subjects who completed a previous pegvaliase study (Phase 2 studies, or 165-301) were eligible for participation. The four parts include:

- Part 1 is an open-label (from 3 to up to 13 weeks) period to establish eligibility for participation in Part 2. Subjects from study 165-301 continued previous dose regimen and subjects from a Phase 2 study were randomized 1:1 to pegvaliase 20 or 40 mg/day upon entry into Part 1. Subjects were assessed every 2 weeks to determine if a mean blood Phe reduction (based on two consecutive assessments) from naïve baseline levels ≥20% and subjects maintained their randomized pegvaliase dose level. These subjects were then eligible for Part 2.
- Part 2 is a randomized, double-blind, placebo-controlled, discontinuation period (8 weeks) to compare blood Phe levels of subjects treated with pegvaliase versus those treated with a placebo. Subjects unable to complete Part 2 due to AEs transitioned to Part 4, the on-going open-label part as tolerated.
- Part 3 is an open-label period (6 weeks) designed to compare the pharmacokinetics of two drug presentations of pegvaliase (vial and syringe versus prefilled syringe).
- Part 4 is an ongoing open-label extension (up to 212 weeks) designed to evaluate the long-term efficacy and safety of pegvaliase. Dose increases up to 60 mg/day per investigator discretion in consultation with the sponsor's medical monitor provided the subject has received a combined total of ≥ 52 weeks of pegvaliase (in this study and previous studies) and has a minimum of 8 weeks at the 40 mg/day dose. Dose reductions may occur if warranted due to AEs or hypophenylalanemia at any time during Part 4.

4 RESPONSES TO CONSULT QUESTIONS

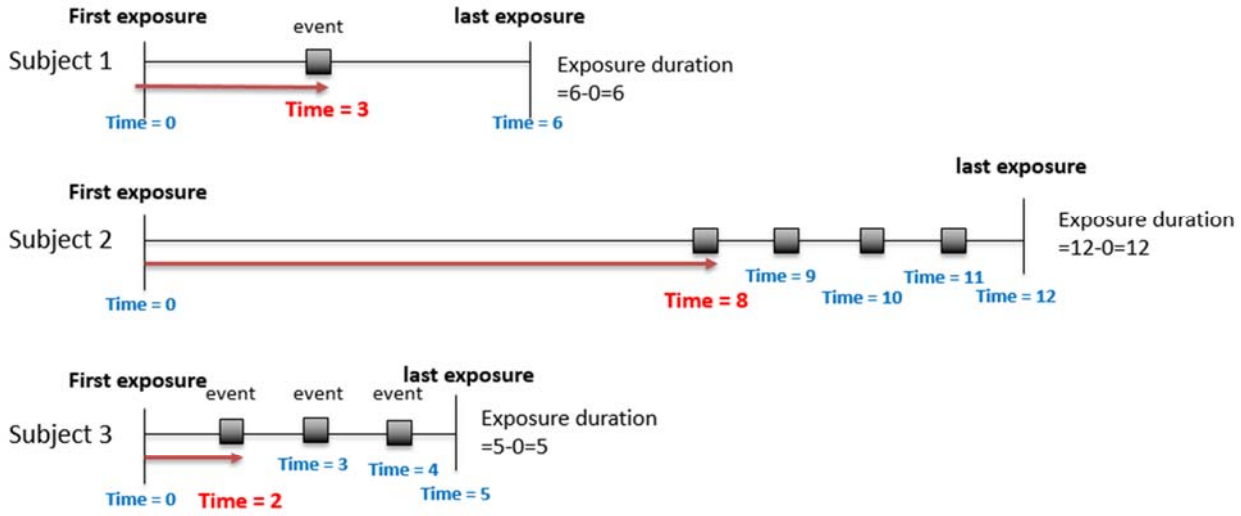
4.1 Appropriateness of using "events/person-years" to adjust for varying durations of exposure in patients

The sponsor used exposure-adjusted event rates (EAER), defined as the total number of events over the total exposure time (events/person-years). Total exposure time was defined as the time from the first dose to the last dose administered across all studies in which the subject was enrolled. Incorporating exposure time to estimate risk is appropriate. Unlike typical clinical trials where all subjects have equal exposure time, this trial included a period that varied in duration.

Another rate that incorporates exposure time is exposure-adjusted incidence rate (EAIR), defined as the number subjects who experienced the event of interest over the exposure time. In this case, the exposure time is defined as the time from first dose until occurrence of the first event of interest. Figure 1 illustrates the EAER and EAIR.

In this trial, subjects continued treatment even after they experienced anaphylaxis. Therefore, some subjects experienced anaphylaxis (or event of interest) multiple times. Because EAER does not censor exposure time at the first occurrence of an event, and counts the number of events, when estimating risk of a reoccurring event, EAER is appropriate. When the risk in terms of number of subjects affected is of interest, EAIR should be presented.

Figure 1. Example to illustrate exposure-adjusted event rate (EAER)and exposure-adjusted incidence rate (EAIR)



$$EAER = \frac{\#of\ events}{Exposure\ time\ of\ subject\ 1 + Exposure\ time\ of\ subject\ 2 + Exposure\ time\ of\ subject\ 3} = \frac{1+4+3}{6+12+5} = 0.35\ Events/Person\text{-}Year$$

$$EAIR = \frac{\#of\ subjects\ with\ event}{Time\ to\ event\ of\ subject\ 1 + Time\ to\ event\ of\ subject\ 2 + Time\ to\ event\ of\ subject\ 3} = \frac{1+1+1}{3+8+2} = 0.23\ Events/Person\text{-}Year$$

Source: Reviewer's illustration

4.2 Best ways to integrate safety analyses (e.g., pooling) in the context of treatment-naïve versus non-treatment naïve patients

The sponsor compared the first 16 weeks before versus after implementation of risk mitigation that includes a heterogeneous group of patients to evaluate the risk mitigation strategies. As discussed in section 3, Study 165-301 comprises induction, titration, and maintenance phases. Subjects initiated pegvaliase in the induction phase. In the titration phase, the dose was titrated up until reaching a stable dose, determined by completing an 8-week phase with at least 80% compliance rate at the same dose level. Subjects then maintained the stable does for at least 2 weeks (maintenance phase) before they transitioned to Study 165-302.

Subjects in the induction and titration phases were treatment-naïve; exposure-status distinguishable from the subsequent phase (maintenance) and study (165-302). Presenting the rate of AEs by study (or the studies combined) might be reasonable if the risk of AEs among treatment-naïve subjects were the same among non-treatment naïve patients. Because we lack information on how exposure status affects safety, we recommend presenting the incidence and the exposure-adjusted event rate of AEs separately for the induction and titration phases from the maintenance phase. The sponsor provided the tables per FDA's request (Table 1).

Table 1. Incidence and exposure-adjusted incidence rate of adverse events (by Preferred Term) by treatment phase

	Induction/Titration Phase N = 285 Treatment Duration Mean = 178 days; Median = 116 days; Range: 1 to 1607 days Total treatment exposure, person-years: 135.4		Maintenance Phase N = 223 Treatment Duration Mean = 739 days; Median = 697 days; Range: 5 to 1561 days Total treatment exposure, person-years: 444.1	
Adverse Events by SMQ/HLT/PT	Incidence [Number of Subjects (%)]	Exposure-Adjusted Event Rate [Number of Events/ Person-Year]	Incidence [Number of Subjects (%)]	Exposure-Adjusted Event Rate [Number of Events/ Person-Year]
Any AE leading to study drug discontinuation	32 (11%)	71 (0.52)	13 (6%)	21 (0.05)
Skin				
Injection site reaction (MedDRA high level term) [a]	4 (1%)	5 (0.04)	1 (<1%)	1 (<0.01)
Generalized skin reaction lasting ≥ 14 Days (Sponsor defined) [b]	5 (2%)	6 (0.04)	1 (<1%)	1 (<0.01)
Pruritus	3 (1%)	3 (0.02)	0 (0%)	0 (0)
Hyperhidrosis	1 (<1%)	1 (0.01)	0 (0%)	0 (0)
Pruritus generalised	1 (<1%)	2 (0.01)	0 (0%)	0 (0)

Source: Table 3 from Sponsor's response, dated March 26, 2018, to FDA's information request

See section 4.3 for an alternative method to present safety information per duration of exposure.

4.3 Alternative approaches to comparing event incidences in trial 165-301, especially during the titration period when the dose was not stable

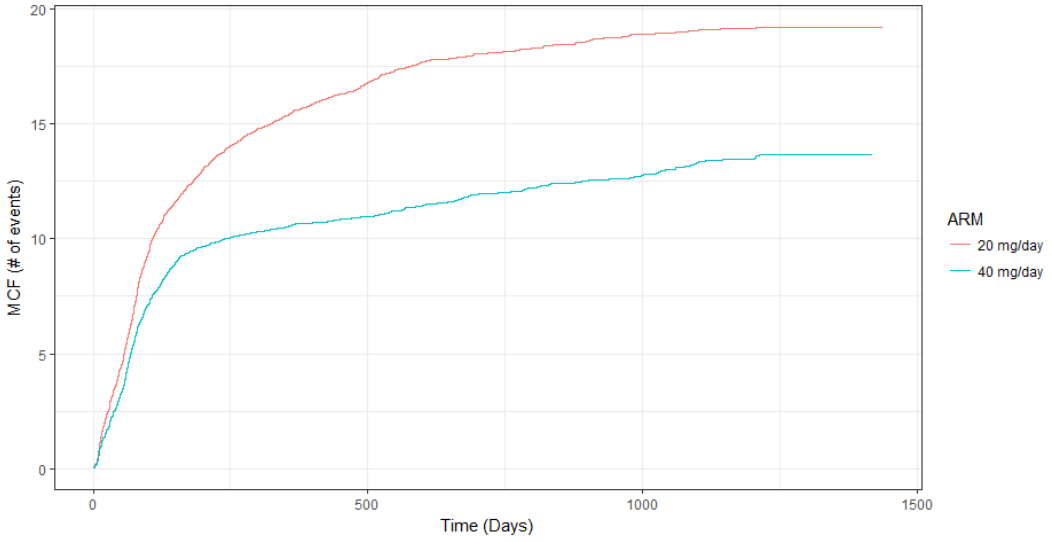
Mean cumulative function (MCF) is a non-parametric approach used to analyze reoccuring AEs. MCF at time t, M(t), is the mean cumulative number of AE of interest in one subject up to time t (Figure 2 and Figure 3). MCF assumes that 1) the sample units represent a simple random sample of the target population; and 2) cumulative history functions of all sample units are statistically independent of their censoring times. Compared with others methods (Table 2), MCF incorporates reoccuring AEs and is presented with respect to exposure time. MCF does not assume constant hazard, and presents the trend of occurrence of AEs relative to exposure time. When the event rate is not constant, exposure adjustments might dilute the treatment effect. Treatment comparisions based on exposure adjusted methods (section 4.1) can be biased when events occur soon after exposure, occur after a latent period, or when event rate decreases over time[1,2]. For example, the exposure adjustment estimates for anaphylaxis events that often occur soon after exposure, might understate the treatment effect.

Figure 2 is the MCF for all AEs recorded in the trials. The 20mg/day and 40mg/day arms included 131, and 130 subjects, and the maximum treatment duration were 1437 days and 1420 days, respectively. By day 500, subjects on the 40mg/day dose experienced a mean of approximately 11 AEs, and subjects on the 20 mg/day dose experienced a mean of approximately 17 AEs.

[1] Siddiqui O. Statistical methods to analyze adverse events data of randomized clinical trials. Journal of biopharmaceutical statistics. 2009 Aug 18;19(5):889-99.
[2] Liu GF, Wang J, Liu K, Snavely DB. Confidence intervals for an exposure adjusted incidence rate difference with applications to clinical trials. Statistics in medicine. 2006 Apr 30;25(8):1275-86.

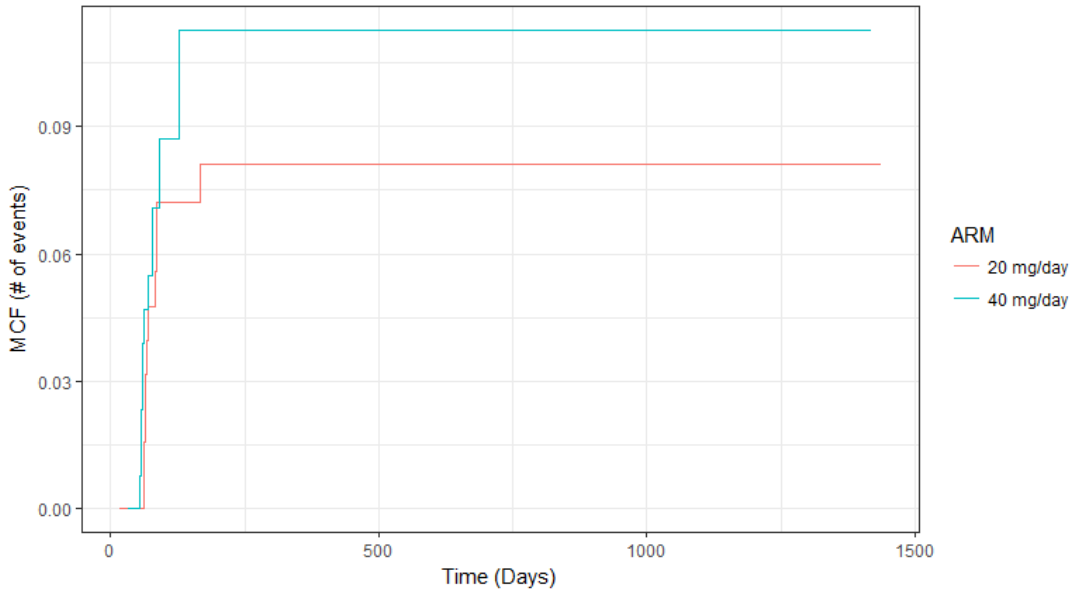
Figure 3 illustrates the MCF for FDA adjudicated anaphylaxis by dose. Subjects on the 40mg/day dose had higher FDA adjudicated anaphylaxis event on average than the 20mg/day dose.

Figure 2. Mean cumulative function of AEs by 20mg/day and 40mg/day in 165-301



Source: Reviewer's analysis

Figure 3. Mean cumulative function of FDA adjudicated anaphylaxis (165-301)



Source: Reviewer's analysis

Table 2. Comparison among incidence, exposure-adjusted rates and mean cumulative function

	Incidence (# of subjects with AEs/ total # of subjects)	Incidence (# of AEs/total # of subjects)	Exposure-Adjusted Incidence Rate	Exposure-Adjusted Event Rate	Mean Cumulative Fucntion
Incorporates reoccuring AEs	No	Yes	No	Yes	Yes
Incorporates exposure time	No	No	Yes	Yes	Yes
Requires constant hazard	*NA*	*NA*	Yes	Yes	No

NA: Not Applicable
Source: Reviewer's table summarized from literature

4.4 Best ways to determine the impact of risk mitigation strategies before and after May 2014

The sponsor implemented four risk mitigation strategies on May 9, 2014. Because all four strategies were implemented simultaneously, we cannot determine the impact of individual stratigies. To assess the impact of all four strategies together, if the measure of impact were collected longitudinally at equal intervals, an interrupted time series analysis might have been applicable. However, the risk mitigation measures were not well defined, nor collected conducive for a model based analysis. Also, having treatment-naïve and non-treatment naïve subjects both included in the population limits the type of analyses possible.

By May 9, 2014, when the sponsor implemented the risk strategies, 91 subjects finished the study; and 52 subjects had started and continued their first 16 weeks of treatment. A total of 118 treatment-naïve subjects enrolled after May 9, 2014. We agree with Division of Biometrics 3's (DB3) suggestion to present the event rate (Table 3) and number of subjects who experience adverse events of interest (Table 4), before and after May 9, 2014, separately; and treatment naïve and conitinuing patients separately. These tables implicitly present the impact of mitigation strategies.

Table 3: Exposure-Adjusted Event rate of special adverse events

	First 16 Weeks Before Risk Mitigation Strategies		First 16 Weeks After Risk Mitigation Strategies (N=52)	First 16 Weeks After Risk Mitigation Strategies (N=118)
	Finished treatment by 5/9/2014 (N=91)	Treatment ongoing (N=52)		
Total exposure duration, person-years				

AEs leading to study drug discontinuation				
Hypersensitivity				
Hypersensitivity AEs based on modified SMQ				
Hypersensitivity AEs based on PT				
AEs with CTCAE Grade ≥ 3				
SAEs				
SAEs assessed by investigator as related to study drug				
SAEs leading to study drug discontinuation				
Anaphylaxis				
Potential anaphylaxis (internal search)				
Adjudicated anaphylaxis (external expert)				
Adjudicated anaphylaxis meeting Brown's severe criteria				

Source: Suggested safety table shells by DB3 and DGEIP email communication dated October 30, 2017

Table 2: Summary of number (%) of patients with special adverse events

	First 16 Weeks Before Risk Mitigation Strategies		First 16 Weeks After Risk Mitigation Strategies (N=52)	First 16 Weeks After Risk Mitigation Strategies (N=118)
	Finished treatment by 5/9/2014 (N=91)	Treatment ongoing (N=52)		
Total exposure duration, person-years				
AEs leading to study drug discontinuation				
Hypersensitivity				
Hypersensitivity AEs based on modified SMQ				
Hypersensitivity AEs based on PT				
AEs with CTCAE Grade ≥ 3				
SAEs				
SAEs assessed by investigator as related to study drug				
SAEs leading to study drug discontinuation				
Anaphylaxis				
Potential anaphylaxis (internal search)				
Adjudicated anaphylaxis (external expert)				
Adjudicated anaphylaxis meeting Brown's severe criteria				

Source: Suggested safety table shells by DB3 and DGEIP email communication dated October 30, 2017

5 Conclusions

The pivotal trials of pegvaliase included phases where treatment exposure duration differed across subjects and dose fluctuated within subject. Subjects continued treatment even after they experienced the safety outcome of interest, anaphylaxis and hypersensitivity adverse events, resulting in subjects experiencing multiple occurrences of the same event.

Because of the complicated study designs and reoccurring events, DGIEP requested assistance in presenting the safety information.

We recommended exposure time adjusted rates to incorporate varying duration of exposure time in the risk measure; and to separate the induction/titration phase from the maintenance phase to assess the drug effect in treatment-naïve and non-treatment naïve subjects. We suggested mean cumulative function to illustrate when subjects experience events with respect to exposure time. Lastly, we identify limitations of the data to assess the impact of the risk mitigation strategies the sponsor implemented mid trial (May 9, 2014). We agreed with Division of Biometric 3's proposal to present event rates by before and after May 9, 2014, and subject exposure status on May 9, 2014.

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/s/

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CLARA KIM
05/14/2018
I concur.

MARK S LEVENSON
05/14/2018

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY BLA REVIEW AND EVALUATION

Application number:	761079
Supporting document/s:	1
Applicant's letter date:	June 30, 2017
CDER stamp date:	June 30, 2017
Product:	Palynziq (pegvaliase)
Indication:	Reduction of blood phenylalanine concentrations in patients with phenylketonuria
Applicant:	Biomarin Pharmaceutical Inc. Novato, California
Review Division:	Gastroenterology and Inborn Errors Products
Reviewer:	Fang Cai, PhD
Supervisor/Team Leader:	David B. Joseph, PhD
Division Director:	Donna Griebel, MD
Project Manager:	Benjamin Vali

Template Version: September 1, 2010

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1 Executive Summary

1.1 Introduction

Palynziq (pegvaliase) is a PEGylated recombinant phenylalanine ammonia lyase (rAvPAL) protein derived from the cyanobacterium *Anabaena variabilis.* PAL catalyzes the conversion of phenylalanine (Phe) to ammonia and trans-cinnamic acid.

Phenylketonuria (PKU) is a rare autosomal recessive disorder characterized by a deficiency in the enzyme phenylalanine hydroxylase (PAH), which is needed for the conversion of phenylalanine (Phe) to tyrosine. Without functional PAH, Phe rapidly accumulates in the body in PKU patients who do not comply with stringent dietary Phe restriction. PKU patients exhibit neurocognitive, neuropsychiatric, and executive functioning deficits due to elevated Phe which is toxic to cells in the brain. The elevated Phe levels inhibit protein synthesis, affect the normal morphology of myelinating proteins, and lead to arrested or delayed development of dendrites and synapses in the cerebral cortex. Palynziq is indicated for reducing blood phenylalanine concentrations in adult patients with phenylketonuria (PKU) who have uncontrolled blood Phe concentrations greater than 600 micromol/L on existing management.

The ENU2 or BTBRenu2 mouse is a homozygous mutant at the phenylalanine hydroxylase gene locus. This mouse model exhibits clinical characteristics similar to those observed in PKU patients, including abnormally high phenylalanine levels (baseline plasma Phe concentrations of 1000 to 2000 µMol/L), hypopigmentation, and cognitive defects. The ENU2 mouse model was used in proof-of-concept studies with pegvaliase.

1.2 Brief Discussion of Nonclinical Findings

Pegvaliase was tested in ENU2 mice, a model of PKU (phenylketonuria). Subcutaneous injection of pegvaliase significantly reduced blood phenylalanine (Phe), a pharmacodynamic effect that was associated with improvement in weight gain, lifespan, and reproductive capability.

In the 26-week rat SC toxicity study with a 17-week interim sacrifice, pegvaliase at ≥ 8 mg/kg twice weekly produced a dose-dependent vacuolation in multiple organs and tissues including renal tubule cells, and histiocytic cells in liver, spleen, testes, adrenal cortex, mesenteric lymph node, and mandibular lymph node. These changes were not reversible. Anti-pegvaliase antibodies did not bind to the cytoplasmic vacuoles in renal tubular epithelium, or in histiocytic cells in spleen and lymph nodes. However, PEG was detected (via IHC using anti-PEG antibodies) in Kupffer cells in liver sinusoids, sinus histiocytes in the mesenteric lymph nodes, and in renal epithelial cells after 26 weeks of treatment. It is likely that vacuolation in histiocytes of multiple organs was related to PEG accumulation, an event that has been reported with other PEGylated therapeutic proteins. The only correlation with the multiple-organ vacuolation was a slight to moderate decrease in liver enzymes (AST, ALP) and urine pH. Thus, the clinical

importance or relevance is unclear. Kidney was considered as the target organ of toxicity, based on the incidence of vacuolation/hypertrophy of renal tubule cells at ≥ 8 mg/kg, which failed to reverse after the 12-week recovery period. The NOAEL was 1 mg/kg twice weekly.

In the 4-week SC toxicity study in monkeys, pegvaliase at ≥ 0.1 mg/kg twice weekly caused minimal to slight vascular degeneration, mainly in the medium-sized, muscular arteries of the following organs: lung, stomach, gallbladder, kidney, colon, pancreas, spleen, and prostate. The vascular degeneration was reversible. No immunohistochemistry was conducted to evaluate whether the vascular degeneration was an immune-mediated response to pegvaliase. The target organs of toxicity were arteries and injection sites. The NOAEL was 0.01 mg/kg based on degeneration of arteries at 0.1 mg/kg and higher.

In the 39-week SC toxicity study in monkeys, pegvaliase at ≥ 3 mg/kg twice weekly produced systemic arteritis, with arterial inflammation present in a wide spectrum of organs without localization to specific sites. Arteritis occurred in small arteries and arterioles in a wide range of organs and tissues (kidney, urinary bladder, pancreas, gallbladder, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, lung, heart, sciatic nerve, lacrimal gland, mandibular lymph node, epididymis, seminal vesicle, ovary, uterus, cervix, and vagina) and in the injection sites. No arterial inflammation was observed at the end of 13-week recovery period. Immune-complex material was detected in affected vessels in examined organs (heart, kidney, and liver) in animals treated at ≥ 3 mg/kg. Pegvaliase and/or its PEG moiety identified by IHC were present in intravascular proteinaceous material, endothelium, interstitial proteinaceous material, and mononuclear cells in the liver and kidney at ≥ 3 mg/kg. The target organs of toxicity were arteries in multiple organs and bone marrow (increased lymphoid nodules). The NOAEL was considered to be 1 mg/kg twice weekly. The drug-induced systemic arteritis was likely related to the vascular accumulation of immune-complex material generated from the immunogenic response to pegvaliase, since immune-complex was detected in the affected vessels. Systemic arteritis was not associated with adverse changes in hematology, clinical chemistry, or histopathology in liver, kidney, or other organs. Arterial inflammation was reversible after treatment was discontinued. Thus, the clinical importance and translatability to humans of systemic arteritis observed in the monkeys is unknown.

In a combined fertility and embryo-fetal development study in rats, pegvaliase at ≥ 8 mg/kg/day significantly reduced the number of corpora lutea and implantations, litter size (20 mg/kg/day only), live fetuses (20 mg/kg/day only), and fetal weights (20 mg/kg/day only). At 20 mg/kg/day, pegvaliase produced decreases in weight gain in females prior to mating, during mating, and during pregnancy (↓up to 64%). The impairment of weight gain was associated with reduced food consumption (↓up to 21.3%). Males at 20 mg/kg/day also showed these effects, but with less severity. Pegvaliase produced a dose-dependent decrease in plasma Phe levels in both sexes. The Phe level was below the low limit of quantification (1 µM) at 20 mg/kg/day. Pegvaliase at ≥ 8 mg/kg/day significantly increased the number fetuses with alterations, but there was no

increase in malformations. Pegvaliase was detected in fetuses in the 20 mg/kg/day group. The NOAEL for male fertility was 20 mg/kg/day, and the NOAEL for female fertility was 8 mg/kg/day based on the decrease in corpora lutea at 20 mg/kg/day. The NOAEL for embryo-fetal development was 8 mg/kg/day, based on the reduced fetal weights at 20 mg/kg/day.

In an embryo-fetal development study in rabbits, subcutaneous administration of 5 mg/kg/day produced a high incidence of external malformations of the head, body, and limbs, and multiple malformations in visceral organs and all regions of the skeletal system (e.g. > 50% incidence of shortened limbs among fetuses and litters). Although the dose which produced malformations also caused clear signs of maternal toxicity (e.g. abortion and premature death in 8% of rabbits, marked impairment of weight gain and food consumption in the surviving females), the malformations are not considered to be secondary to maternal toxicity based on their severity and high incidence. Other adverse effects observed at 5 mg/mg/day included significant increases in late resorptions, post-implantation loss, and the number of does with any resorptions. Reductions in male and female fetal weight and the number live male fetuses were also observed. The observed reduction in maternal weight gain during days 7-29 of gestation was 23% and 59% in the 2 and 5 mg/kg/day groups, respectively. These values represent the mean reduction observed in the three treatment periods used in this study (i.e. GD 7-12, 11-16 and 15-20). During treatment (days 7-20 of gestation), plasma Phe levels in the 2 and 5 mg/kg/day groups were decreased by an average of 90% and 99%, respectively. The NOAEL for embryo-fetal developmental toxicity was 2 mg/kg/day. The maternal NOAEL was not identified (< 2 mg/kg/day), due to the reductions in weight gain and food consumption in the 2 mg/kg/day group.

In a pre-/postnatal development study in rats, pegvaliase at 20 mg/kg/day produced significant decreases in maternal body weight gain (↓up to 25.8%) and food consumption (↓up to 33.4%). At 20 mg/kg/day, pegvaliase significantly reduced viability index (↓15.5%), lactation index (↓9%), pup survival during postpartum days 1-4 (↓up to 28.7%), and pup weight during lactation (↓up to 28.3%). The offspring from the 20 mg/kg/day group had significant decreases in body weight, body weight gain, and food consumption (males only), and showed a delay in sexual maturation. No pegvaliase was detected in the offspring. However, pegvaliase was detected in milk at all doses (2, 8, and 20 mg/kg/day). There was a dose-dependent reduction in maternal plasma phenylalanine level. The apparent NOAEL for developmental effects was 8 mg/kg/day. However, this conclusion is only preliminary, given that this study lacked sufficient testing to provide an adequate evaluation of postnatal development. Learning and memory in the offspring (F1 generation) were evaluated through the Passive Avoidance and M-Maze Water Maze tests, which showed no effects of pegvaliase. However, no other tests were conducted to evaluate behavior, motor activity, sensory or sensorimotor functions, or reflex development. The adverse effects in offspring at 20 mg/kg/day may have been secondary to maternal toxicity. The NOAEL for maternal toxicity was 8 mg/kg/day. This study should be repeated with the inclusion of tests to evaluate the effects on behavior, motor activity, sensory or sensorimotor functions, and reflex development.

Pegvaliase produced a dose-dependent decrease in plasma Phe levels in rats and rabbits in the reproductive and developmental studies. At the highest doses tested (i.e. 20 mg/kg/day in rats and 5 mg/kg/day in rabbits), Phe was reduced by 99% in both species. Maternal toxicity was more prominent in rabbits, based on the incidence of mortality, abortion, and weight loss in 8% of rabbits treated with 5 mg/kg/day. However, the inhibition of maternal weight gain was similar in the high-dose rats (↓50%) and the surviving high-dose rabbits (↓59%). Rabbit fetuses in the high-dose group showed a high incidence of external malformations of the head, body and limbs, multiple soft tissue malformation, and skeletal malformations in all regions, whereas no increase in malformations were observed in the rat study. The incidence of malformations in rabbits was markedly increased among fetuses and litters. The Sponsor did not conduct studies to investigate the mechanism for the adverse developmental effects in rabbits, although the Sponsor proposed that these effects can be attributed to abnormally low levels of plasma Phe due to the pharmacologic activity of pegvaliase.

The Division of Gastroenterology and Inborn Errors Products and Office of Drug Evaluation III have requested a study to investigate the potential involvement of maternal phenylalanine depletion in the malformations caused by pegvaliase in pregnant rabbits. This request is based on the concern raised by the high incidence of malformations in rabbit fetuses which occurred at a small multiple of human exposure (7.5-fold based mg/kg), and the need to address this concern by providing additional information for inclusion in the Pregnancy subsection of the label.

1.3 Recommendations

1.3.1 Approvability

There are no approvability issues from a nonclinical viewpoint.

1.3.2 Additional Nonclinical Recommendations

1. Recommendations for labeling changes are shown in the following section.

2. The Sponsor should conduct an additional pre-/postnatal development study in rats using a set of testing methods that is sufficient to evaluate postnatal development, including tests for effects on behavior, motor activity, sensory or sensorimotor functions, and reflex development.

1.3.3 Labeling

Established Pharmacologic Class Text Phrase

Sponsor's Proposed Version:

Palynziq is a (b) (4) phenylalanine-metabolizing enzyme indicated to reduce blood phenylalanine in adult patients with phenylketonuria who have uncontrolled blood phenylalanine levels > 600 µmol/L on existing management.

Evaluation:

Palynziq (Pegvaliase) is a first-in-class NME. The Sponsor has proposed the term "(b) (4) phenylalanine-metabolizing enzyme" as the established pharmacologic class for Palynziq (Pegvaliase).

Phenylalanine ammonia lyases (PALs) are members of a superfamily of ammonia lyases that includes histidine ammonia lyase and tyrosine ammonia lyase. PAL, which is found in plants, fungi and bacteria, catalyzes the deamination of L-phenylalanine to trans-cinnamic acid and ammonia (Hyun MW et al. Mycobiology 2011, 39(4): 257-265; Moffitt MC et al. Biochemistry. 2007, 46(4): 1004–1012). In plants, this is the first step in biosynthesis of plant phenylpropanoids. In fungi and bacteria, PAL is associated with the biosynthesis of the secondary metabolites enterocin, cinnamide and 3,5-dihydroxy-4-isopropylstilbene (Hyun MW et al. Mycobiology 2011, 39(4): 257-265; Moffitt MC et al. Biochemistry. 2007, 46(4): 1004–1012; Xiang, L et al. J. Biol. Chem. 2002, 277, 32505−32509).

Pegvaliase is a recombinant cyanobacterium anabaena variabilis PAL (rAv-PAL). Two point mutations (Cys503Ser and Cys565Ser) have been introduced into the native AvPAL sequence to mitigate potential aggregation through reduced intramolecular disulfide formation. To reduce the immune response to PAL *in vivo*, rAvPAL protein is conjugated with linear N-hydroxysuccinimide methoxypolyethylene glycol (NHS-PEG). Pegvaliase reduced blood phenylalanine levels in a PKU mouse model and in normal animals (rats, rabbits and monkeys) in pharmacology and toxicology studies conducted by the Sponsor. (b) (4)

(b) (4)

Recommended Version:

Palynziq is a phenylalanine-metabolizing enzyme indicated to reduce blood phenylalanine in adult patients with phenylketonuria who have uncontrolled blood phenylalanine levels > 600 µmol/L on existing management.

Sponsor's Proposed Version:

8.1 Pregnancy

(b) (4)

(b) (4)

Animal Data

(b) (4)

(b) (4)

Evaluation:

We do not concur with the Sponsor's description of the results from the developmental studies in rats and rabbits, therefore major revisions are needed (b) (4) (b) (4) (b) (4) In the recommended version below, the rat AUC_{0-72} values from the end of the dosing period in the 26-week rat toxicology study are used for calculation of rat/human AUC_{0-72} ratios. The rat AUC_{0-72} was 1,036,762 ng•hr/ml and 4,787,043 ng•hr/ml at 8 and 20 mg/kg twice per week, respectively (AUC for 20 mg/kg was extrapolated from 25 mg/ kg used in the 26-week study). The estimated human AUC_{0-72} is 740,349 ng•hr/ml, based on PK data in PKU patients treated with 40 mg/day, the maximum recommended dose. The AUC_{0-72} in humans was derived from the AUC_{0-24} (246,783 ng•hr/ml) reported for PKU patients.

We do not recommend the use of AUC ratios to express the rabbit to human exposure multiples in the rabbit embryo-fetal development study. TK measurement in rabbits was conducted only in the dose-range finding study in pregnant rabbits, which showed profound changes in AUC after only a few days of dosing. These changes were likely due to the formation of anti-drug antibodies, which resulted in highly disproportional AUC values in the two dose groups; the AUC_{last} in the 5 mg/kg/day females was 531 times the AUC_{last} in the 2 mg/kg/day females. Therefore, the use of rabbit to human exposure comparison based on mg/kg is an acceptable and reasonable alternative for the rabbit embryo-fetal development study. This approach for animal to human scaling is recommended for therapeutic proteins with molecular weight > 100,000 Daltons (FDA Guidance, "*Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers*"). The human dose of 0.67 mg/kg should be used for calculation of the rabbit to human exposure ratio, based on the maximum recommended dose of 40 mg/day and an assumed bodyweight of 60 kg.

The inclusion of (b) (4) in the Sponsor's proposed version is not appropriate for labeling. Therefore, this should be deleted. The recommended version below was developed in collaboration with the Maternal Health team (Catherine Roca and Miriam Dinatale).

Recommended Version:

Risk Summary

Based on findings in studies from animals without PKU, Palynziq may cause fetal harm when administered to a pregnant woman. Limited available data with pegvaliase-pqpz use in pregnant women are insufficient to inform a drug-associated risk of adverse developmental outcomes. There are risks to the fetus associated with poorly controlled phenylalanine levels including increased risk for miscarriage, major birth defects (including microcephaly, major cardiac malformations), intrauterine fetal growth retardation, and future intellectual disability with low IQ; therefore, phenylalanine levels should be monitored during pregnancy *(see Clinical Considerations and Data).* A reproduction study with pegvaliase-pqpz in rabbits demonstrated a high incidence of malformations throughout the skeletal system, and in kidneys, lungs, and eyes. Embryo-fetal toxicity (increased resorptions and reduced fetal weight) was also observed. These effects occurred at 7.5 times the maximum recommended daily dose, and were associated with strong signs of maternal toxicity, including marked reductions in weight gain and food consumption, and death. A reproduction study in rats demonstrated an increase in skeletal variations, with no malformations observed. The effects in rats occurred at (b) (4) times the maximum recommended daily dose. In a pre-/postnatal development study in rats, pegvaliase-pqpz produced decreases in survival of offspring during lactation, pup weight, and litter size, and delayed sexual maturation of offspring when administered daily at (b) (4) times the maximum recommended daily dose. The effects on rat embryo-fetal and post-natal development were associated with maternal toxicity. Advise pregnant women of the potential risk to a fetus.

There is a pregnancy pharmacovigilance program for Palynziq. If Palynziq is administered during pregnancy, or if a patient becomes pregnant while receiving Palynziq or within one month following the last dose of Palynziq, healthcare providers should report Palynziq exposure by calling 1- (b) (4) .The estimated background risk of major birth defects and miscarriage for women with PKU with blood phenylalanine greater than (b) (4) , respectively. All pregnancies have a background risk of major birth defect, loss, or other adverse outcomes. In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2 to 4% and 15 to 20%, respectively.

Clinical Considerations

Disease-Associated Maternal and/or Embryo/Fetal Risk
Uncontrolled blood phenylalanine concentrations before and during pregnancy are associated with increased risk of adverse pregnancy outcomes. To reduce the risk of hyperphenylalaninemia-induced teratogenic effects, target blood phenylalanine concentrations 120 to 360 micromol/L during pregnancy and 3 months before conception *[see Dosage and Administration (2.2)].*

Dose adjustments during pregnancy and the postpartum period
Phenylalanine levels below 30 micromol/L may be associated with adverse fetal outcomes. Monitor blood phenylalanine levels during pregnancy and adjust the dosage of Palynziq or modify dietary protein and phenylalanine intake to avoid blood

phenylalanine concentrations below 30 micromol/L *[see Dosage and Administration (2.1) and (2.2)].*

Data
Human Data

Uncontrolled Maternal PKU: Available data from the Maternal Phenylketonuria Collaborative Study on 468 pregnancies and 331 live births in PKU-affected women demonstrated that uncontrolled phenylalanine concentrations above 600 micromol/L are associated with an increased risk for miscarriage, major birth defects (including microcephaly, major cardiac malformations), intrauterine fetal growth retardation, and future intellectual disability with low IQ.

Limited data from case reports of Palynziq use in pregnant women are insufficient to determine a drug-associated risk of adverse developmental outcomes.
Animal Data

All developmental toxicity studies were conducted in normal animals (rats and rabbits), in which pegvaliase treatment produced a dose-dependent reduction in maternal plasma phenylalanine concentrations. At doses which produced maternal toxicity and/or effects on embryo-fetal development, the maternal plasma phenylalanine concentrations were markedly reduced compared to the control group. The contribution of maternal phenylalanine depletion to the incidence of embryo-fetal developmental effects was not evaluated.

Subcutaneous administration of 5 mg/kg/day pegvaliase-pqpz (7.5 times the maximum recommended daily dose based on mg/kg) in pregnant rabbits during the period of organogenesis produced embryo-lethality (increased resorptions), marked reduction in fetal weight, and malformations. The malformations included multiple external abnormalities of the head, body and limbs, multiple soft tissue malformations (reduced size or absence of kidneys, diaphragm hernia, corneal opacity, discoloration or reduced size of eyes, and reduced size of lungs) and multiple skeletal malformations of the craniofacial bones, vertebrae, sternebrae, ribs, pelvis, limbs, and digits. An increase in variations and delayed ossification was also observed in all skeletal regions. The adverse developmental effects were associated with maternal toxicity, as indicated by marked impairment of weight gain and food consumption. Deaths associated with weight loss and abortion occurred in 8% of rabbits treated with 5 mg/kg/day pegvaliase-pqpz.

Subcutaneous administration of 2 mg/kg/day pegvaliase-pqpz (3 times the maximum recommended daily dose based on mg/kg) in pregnant rabbits had no adverse effects on embryo-fetal development. Systemic exposure to pegvaliase was detected in fetuses from rabbits treated with 2 or 5 mg/kg/day.

Pegvaliase-pqpz increased fetal alterations when administered daily in rats at doses of 8 mg/kg subcutaneously and higher (b) (4) times the human steady-state AUC at the

maximum recommended daily dose) during a 28-day premating period, mating, and through the period of organogenesis. The fetal alterations were limited to variations such as cervical ribs, bifid centra of lumbar and thoracic vertebrae, and incomplete ossification of squamosal bones, frontal bones, lumbar vertebra arch, and ribs. Daily administration of 20 mg/kg subcutaneously (b) (4) times the human steady-state AUC at the recommended maximum daily dose) produced reductions in litter sizes and fetal weights, which was associated with maternal toxicity (decreased body weight, ovarian weight, and food consumption). The decrease in litter sizes at 20 mg/kg subcutaneously was secondary to reductions in corpora lutea and implantations. Systemic exposure to pegvaliase-pqpz was detected in fetuses from rats treated with 20 mg/kg ((b) (4) times the human steady-state AUC at the recommended maximum daily dose). Subcutaneous administration of 2 mg/kg/day pegvaliase-pqpz (less than (b) (4) the human steady state AUC at the maximum recommended daily dose) in pregnant rats had no adverse effects on embryo-fetal development.

Pegvaliase-pqpz decreased the pup weight, litter size, survival of offspring during lactation, and delayed sexual maturation of offspring when administered daily in rats at 20 mg/kg subcutaneously ((b) (4) times the human steady-state exposure at the recommended maximum daily dose), with dosing starting before mating and continuing through lactation. The effects in offspring were associated with maternal toxicity. No effects in offspring were observed at 8 mg/kg/day subcutaneously ((b) (4) times the human steady-state exposure at the recommended maximum daily dose). This study lacked a complete evaluation of neurobehavioral development in offspring; however, no effects of pegvaliase-pqpz were noted in tests for learning and memory.

Sponsor's Proposed Version:

8.2 Lactation

Risk Summary

(b) (4)

Evaluation:

The inclusion of an animal to human exposure comparison is not recommended for this subsection. The decrease in pup weight and survival observed in the pre-/postnatal study in rats should be stated. The recommended version below was developed in collaboration with the Maternal Health team (Catherine Roca and Miriam Dinatale).

Recommended Version:

Risk Summary
There are no data on the presence of pegvaliase in human milk, the effects on the breastfed infant, or the effects on milk production. A pre-/postnatal study in rats showed that pegvaliase-pqpz is present in rat milk and that administration of pegvaliase-pqpz during lactation decreased pup weight and survival. However, systemic absorption of pegvaliase was not detected in the rat pups *[see Use in Specific Populations (8.1)].* Palynziq may cause low phenylalanine levels in human milk *(see Clinical Considerations).* The developmental and health benefits of breastfeeding should be considered along with the clinical need for Palynziq and any potential adverse effect on the breastfed infant from Palynziq or from the underlying condition.

Clinical Considerations
Monitor phenylalanine levels in a breastfeeding woman.

Sponsor's Proposed Version:

12.1 Mechanism of Action

(b) (4)

Pegvaliase is a phenylalanine ammonia lyase enzyme that converts phenylalanine to ammonia and *trans*-cinnamic acid (b) (4) It substitutes for the deficient PAH enzyme activity and reduces blood phenylalanine levels in the body.

Evaluation:

Recombinant phenylalanine ammonia lyase (rAv-PAL) is derived from the cyanobacterium *Anabaena variabilis*, expressed in *E. coli*. The rAvPAL is conjugated to N–hydroxysuccinimide (NHS) active ester-methoxy-polyethylene glycol (NHS-PEG) to produce the drug substance, rAvPAL-PEG (pegvaliase). Thus, pegvaliase is a PEGylated phenylalanine ammonia lyase enzyme. Phenylalanine ammonia lyase converts phenylalanine to ammonia and trans-cinnamic acid. Ammonia is metabolized to urea in the liver, and trans-cinnamic acid is excreted in urine. Although phenylalanine metabolism by pegvaliase and PAH yields different products (i.e. ammonia and trans-cinnamic acid from pegvaliase, tyrosine from PAH), pegvaliase is clearly effective in reducing blood phenylalanine levels. Thus, the Sponsor's statement that pegvaliase "*substitutes for the deficient PAH enzyme activity and reduces blood phenylalanine levels in the body*" is acceptable. The recommended version below was developed by the Clinical Pharmacology team.

Recommended Version:

When administered to humans, pegvaliase-pqpz converts phenylalanine to ammonia and *trans*-cinnamic acid which are excreted in the urine. PKU is caused by deficiency of phenylalanine hydroxylase (PAH), an enzyme which catalyzes the hydroxylation of phenylalanine to tyrosine in the presence of the cofactor tetrahydrobiopterin (BH4). Due to PAH deficiency in patients with PKU, phenylalanine accumulates to toxic levels in the body. In patients with PKU, pegvaliase substitutes for the deficient PAH enzyme activity and reduces blood phenylalanine concentrations.

Sponsor's Proposed Version:

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

(b) (4)

(b) (4) Based on the mechanism of action, pegvaliase is not expected to be tumorigenic.

(b) (4)

Evaluation:

Carcinogenicity and genotoxicity studies have not been performed with pegvaliase. We concur with the Sponsor's statement, "*Based on the mechanism of action, pegvaliase is not expected to be tumorigenic*".

(b) (4)

(b) (4) In addition, AUC data is not available from the combined fertility and embryo-fetal developmental study in rats. However, AUC data for doses of 8 and 25 mg/kg twice weekly is available from the 26-week SC toxicity study in rats. The rat AUC_{0-72} was 1,036,762 ng•hr/ml and 4,787,043 ng•hr/ml at 8 and 20 mg/kg twice per week, respectively (AUC for 20 mg/kg was extrapolated from 25 mg/kg used in the 26-week study). Therefore, this labeling section should include the rat to human AUC ratio. The estimated human AUC_{0-72} is 740,349 ng•hr/ml, based on PK data in PKU patients treated with 40 mg/day, the maximum recommended dose. The AUC_{0-72} in humans was derived from the AUC_{0-24} (246,783 ng•hr/ml) reported for PKU patients.

Recommended Version:

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenicity and genotoxicity studies have not been performed with pegvaliase-pqpz. Based on the mechanism of action, pegvaliase is not expected to be tumorigenic.

Pegvaliase-pqpz produced impaired fertility in female rats at 20 mg/kg/day subcutaneously (b) (4) times the human steady-state exposure at the maximum recommended daily dose), as indicated by decreases in corpora lutea, implantations, and litter size. These effects were associated with toxicity (decreased body weight, ovarian weight, and food consumption). No effects on mating or fertility were observed in female rats at 8 mg/kg/day given subcutaneously ((b) (4) times the human steady-state exposure at the maximum recommended daily dose) or in male rats at 20 mg/kg/day given subcutaneously.

Sponsor's Proposed Version:

13.2 Animal Toxicology and/or Pharmacology

(b) (4)

Evaluation:

The Sponsor did not provide systemic exposure margins associated with vacuolation in rats and arteritis in monkeys in the respective chronic toxicity studies. The animal to human AUC multiples should be included. The rat AUC_{0-72} was 158,404 ng•hr/ml at 8 mg/kg twice weekly in the 4-week SC toxicity study, and 1,036,762 ng•hr/ml at 8 mg/kg twice weekly in the 26-week SC toxicity study (both values were measured at the end of the dosing period). The lower AUC observed in the 4-week toxicity study in rats should be used for calculation of the rat to human exposure ratio. For systemic arteritis observed in the 39-week SC toxicity study in monkeys, the AUC_{0-72} value at the end of the dosing period was 2,334,176 ng•hr/ml at 3 mg/kg twice weekly.

The estimated human AUC_{0-72} is 740,349 ng•hr/ml, based on PK data in PKU patients treated with 40 mg/day, the maximum recommended dose. The AUC_{0-72} in humans was derived from the AUC_{0-24} (246,783 ng•hr/ml) reported for PKU patients.

(b) (4)

The issues described above are addressed in the recommended version below, which also includes other revisions.

Recommended Version:

13.2 Animal Toxicology and/or Pharmacology

In rats, dose-dependent vacuolation with pegvaliase-pqpz treatment was observed in the 4- and 26-week repeat dose toxicity studies at doses ≥ 8 mg/kg twice weekly subcutaneously (b) (4)). Vacuolation occurred in multiple organs and tissues, including renal tubule cells and in histiocytic cells in liver, spleen, testes, adrenal cortex, mesenteric lymph node, and mandibular lymph node. Vacuolation in histiocytes of the affected organs and tissues persisted after cessation of treatment. The vacuolation observed in these studies was not associated with any organ related toxicities as determined by clinical chemistry/urinalysis and histopathological examination. The clinical significance of these findings and functional consequences are unknown.

In the 39-week repeat dose toxicity study in monkeys, pegvaliase-pqpz at ≥ 3 mg/kg twice weekly subcutaneously (3 times the human steady state AUC at the maximum recommended daily dose) produced systemic arteritis involving small arteries and arterioles in a wide range of organs and tissues (kidney, urinary bladder, pancreas, gallbladder, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, lung, heart, sciatic nerve, lacrimal gland, mandibular lymph node, epididymis, seminal vesicle, ovary, uterus, cervix, and vagina) and in subcutaneous injection sites. Arteritis

was likely due to the immune-mediated response (e.g., immune complex deposition in blood vessels) associated with chronic administration of a foreign protein to the animals. The incidence and severity of systemic arteritis was dose dependent. The vascular inflammation observed in this study was not associated with any organ related toxicities as determined by clinical pathology parameters (hematology, clinical chemistry, and urinalysis) and histopathological examination.

2 Drug Information

2.1 Drug

CAS Registry Number (Optional)

Generic Name: pegvaliase-pqpz

Code Name: BMN 165

Chemical Name: recombinant *Anabaena variabilis* phenylalanine ammonia lyase-PEG (rAvPAL–PEG)

Molecular Formula/Molecular Weight:

Recombinant *Anabaena variabilis* phenylalanine ammonia lyase (rAvPAL) is a homotetramer with a calculated molecular weight per monomer of 61,719 Da (based on peptide sequence). rAvPAL-PEG drug substance is a conjugate of rAvPAL with linear polyethylene glycol (PEG) of 20 kDa molecular mass. The PEG is linked to the rAvPAL via the ε–amine groups of lysine side-chains or to the N–terminus of each peptide monomer. rAvPAL-PEG contains (b) (4) strands of PEG per rAvPAL monomer. The total molecular weight of rAvPAL-PEG is approximately 1000 kDa.

Structure or Biochemical Description:

rAvPAL is derived from the cyanobacterium Anabaena variabilis, expressed in *E. coli*. (b) (4) The rAvPAL was conjugated to N–hydroxysuccinimide (NHS) active ester-methoxy-polyethylene glycol (NHS-PEG) to produce the drug substance, rAvPAL-PEG.

Pharmacologic Class: a phenylalanine-metabolizing enzyme

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 76269

2.3 Drug Formulation

The drug product (DP) is supplied as a sterile, preservative-free solution in a single use, glass prefilled syringe (PFS) with a staked needle for subcutaneous injection. Three PFS dose strengths (2.5 mg/0.5 ml, 10 mg/0.5 ml, and 20 mg/ml) of pegvaliase are available, and the product composition for each strength is summarized in the table below (taken from the Sponsor' submission).

Table 1: Composition of Pegvaliase Drug Product

Component	Quality Standard	Function	Composition[1]			
			2.5 mg[2]	10 mg[2]	20 mg[2]	Concentration
Pegvaliase	In-house Standard[3]	Active Ingredient	2.5 mg	10 mg	20 mg	(b) (4)
Tromethamine	USP/Ph. Eur	(b) (4)	(b) (4)			
Tromethamine –HCl	Manufacturer's CoA[7]					
Sodium Chloride	USP/Ph. Eur	Tonicity Agent				
Trans-cinnamic acid	Not Applicable[8]	(b) (4)	0.07 mg	0.07 mg	0.15 mg	
Water for Injection (qs)	USP/Ph. Eur		0.50 mL	0.50 mL	1.00 mL	

USP: United States Pharmacopeia; Ph. Eur: European Pharmacopeia

[1] The composition provided is for the delivered volume of pegvaliase DP and excludes syringe overfill amounts.

[2] Protein concentrations refer to the protein portion, (b) (4)

[3] Qualified according to procedure described in Section 2.3.S.5.

(b) (4)

[7] There is no applicable monograph for tromethamine-HCl. This excipient is GMP manufactured and released per the manufacturer's certificate of analysis.

(b) (4)

2.4 Comments on Novel Excipients

Trans-cinnamic acid is not included in the FDA Inactive Ingredient database. However, trans-cinnamic acid was an ingredient in the dosing formulations (test article and control article) in all of the nonclinical safety studies. The trans-cinnamic acid concentration

used in the animal formulations (1 mM) was identical to the concentration proposed for the highest strength for pegvaliase. More importantly, the maximum potential daily dose of trans-cinnamic acid is only 300 mcg (5 mcg/kg/day in a 60-kg patient), based on the maximum recommended dose of 40 mg pegvaliase/day. In the 26-week rat toxicity study, all groups treated with pegvaliase or vehicle received trans-cinnamic acid at 375 mcg/kg/dose (HED = 60 mcg/kg). In the 39-week monkey toxicity study, all groups treated with pegvaliase or vehicle received trans-cinnamic acid at 150 mcg/kg/dose (HED = 48 mcg/kg). Given that the tested HEDs for trans-cinnamic acid in the chronic toxicity studies in rats and monkeys are high multiples of the maximum potential human dose (5 mcg/kg/day), trans-cinnamic acid is considered as qualified at the proposed levels in the drug product. It should also be noted that trans-cinnamic acid is a direct product of the enzymatic activity of pegvaliase on its substrate, phenylalanine. However, the systemic exposure to trans-cinnamic acid was not measured in any of the nonclinical studies.

2.5 Comments on Impurities/Degradants of Concern

The administration of pegvaliase is expected to result in exposure to chemicals and elements that leach from the pre-filled syringe or from manufacturing system components. The estimated maximum exposure to leaching chemicals and elements (leachables) with each daily SC injection of pegvaliase is the following: (b) (4)

(b) (4)

(b) (4) The toxicological risk assessment for the potential exposure to these leachables and metal elements is presented in section 12 (Appendix/Attachments). There is no safety concern for the potential exposure to these leachables and metal elements in PKU patients receiving the maximum daily dose of 40 mg pegvaliase SC.

2.6 Proposed Clinical Population and Dosing Regimen

The proposed indication for pegvaliase is to reduce blood phenylalanine (Phe) levels in adult patients with phenylketonuria who have uncontrolled blood Phe levels > 600 µmol/L on existing management. The recommended maximum dose is 40 mg per day via SC injection.

2.7 Regulatory Background

Pegvaliase was developed under IND 76269.

3 Studies Submitted

3.1 Studies Reviewed

Studies	Report #
PHARMACOLOGY	
Pharmacology Study Evaluating Plasma Phe Levels in ENU2 Mice Following Subcutaneous Administration of AvPAL and PEGylated AvPAL	0164-06-019
Evaluation of Plasma Phe Levels Following Subcutaneous Administration of PEGylated AvPAL variant in Naïve ENU2 Mice	0165-06-057
Evaluation of Changes in Neuropathology Following Subcutaneous Administration of BMN 165 to Female BTBR*Pah*enu2 Mice for 4, 8, or 12 Weeks	BMN165-14-044
Dose Range Reduction of rAvPAL-PEG for 16 Weeks with a 2 Week Recovery Period in BTBRPahenu2 Mice	0165-07-001
Assessment of rAvPAL-PEG Dose Ranging Studying FemaleBTBRPahenu2 Mice	0165-07-029 0165-08-005
Assessment of Reproduction in Female BTBRPahenu2 Mice Administered rAvPAL-PEG	0165-08-027
Assessment of rAvPAL-PEG Dose Range Administration for Stabilization of Plasma Phe in Growing Female BTBRPahenu2 Mice	0165-09-068 0165-10-008
SAFETY PHARMACOLOGY	
Neurological Evaluation Study of rAvPAL-PEG in Rats	0165-07-004
Effects of rAvPAL-PEG on Respiratory Functions in SD Rats Using the Head-Out Body Plethysmography Model	0165-07-005
Effects of rAvPAL-PEG on Cardiovascular System Conscious Telemetry-Instrumented Male Monkeys	0165-07-006
PHARMACOKINETICS	
60-Day Dose Escalation Pharmacokinetic Study of PEGylated R91K RtPAL (Phenylalanine Ammonia Lyase) and PEGylated AvPAL in Rats	0164-06-042
Single-Dose Pharmacokinetics/Pharmacodynamics of rAvPAL-PEG in Homozygote (-/-) Male BTBRPahenu2 Mice	0165-07-028
Comparative Single-Dose Pharmacokinetics/ Pharmacodynamics for Phase 2 (b) (4) rAvPAL-PEG in Homozygote (-/-) Male and Female BTBRPahenu2 Mice	0165-09-069 (Males) 0165-09-070 (Females)
Repeat-Dose Pharmacodynamics and Pharmacokinetics of rAvPAL-PEG Administered Subcutaneously Once-Daily for 36 Days in Homozygote (-/-) Male BTBRPahenu2 Mice	0165-08-006

rHuPH20/rAvPAL-PEG Testing in Rats Summary Report	BMN165-11-018
TOXICOLOGY	
Single Dose Toxicity	
Single-Dose Subcutaneous or Intravenous Injection Toxicity and Toxicokinetic Study with rAvPAL-PEG in Rats with a 2-Week Recovery Period	0165-07-003
Single-Dose Subcutaneous Injection Toxicity and Toxicokinetic Study with rAvPAL-PEG in Cynomolgus Monkeys With a 3-Week Recovery Period	0165-07-007
Repeat-Dose Toxicity	
4-Week Subcutaneous Injection Toxicity and Toxicokinetic Study with rAvPAL-PEG in Rats with a 2- Week Recovery Phase	0165-07-009
26-Week Subcutaneous Injection Toxicity and Toxicokinetic Study with a 12-Week Recovery Period and a 17-Week Interim Euthanasia with a 4-Week Recovery Period with rAvPAL-PEG in Rats	0165-08-019
4-Week Subcutaneous Injection Toxicity and Toxicokinetic Study with rAvPAL-PEG in Cynomolgus Monkeys with a 4-Week Recovery Period Recovery	0165-07-008
39-Week Subcutaneous Injection Toxicity and Toxicokinetic Study with rAvPAL-PEG in Cynomolgus Monkeys with a 13-Week Recovery Phase	0165-07-030
Evaluation of Ependymal Cell Vacuolation in Brains from Rats and Monkeys Treated Twice Weekly with Subcutaneous Injections of rAvPAL-PEG in Previous Chronic Toxicology Studies	BMN165-13-047
Development and Validation of an Immunohistochemical Method for the Semi Quantitative Evaluation of Anti-PEG antibody and Anti-rAvPAL-PEG Antibody Binding in Formalin Fixed Paraffin Embedded Rat Tissue	BMN165-13-068
Analysis of Rat Tissues from Toxicology Studies for Anti-PEG and Anti-rAvPAL-PEG using Immunohistochemistry (IHC)	BMN165-13-069
The Immunohistochemical Analysis in Rat Tissues in a 26-Week Subcutaneous Injection Toxicity and Toxicokinetic Study with a 12-Week Recovery Period and a 17-Week Interim Euthanasia with a	16-RS-357 165-08-019

4-Week Recovery Period with rAvPAL PEG in Rats	
REPRODUCTIVE TOXICITY	
Fertility and Embryo-fetal Development Study of rAvPAL-PEG Administered by Subcutaneous Injection in Rats	BMN165-12-037
A Dose Range-finding Embryo-fetal Development Study of rAvPAL-PEG by Subcutaneous Injection in Rabbits	BMN165-11-027
Embryo-fetal Development Study of rAvPAL-PEG Administered by Subcutaneous Injection in Rabbits	BMN165-12-036
Development and Peri-/Post-natal Reproduction Study of Subcutaneous rAvPAL-PEG in Rats, Including a Postnatal Behavioral/Functional Evaluation	BMN165-14-013

3.2 Studies Not Reviewed

Report Number	Report Title
BAS-VR-06-001	Detection of IgG Antibodies Specific to Recombinant Phenylalanine Ammonia Lyase in Mouse Serum
BAS-VR-06-002	Detection of activity neutralizing antibodies to rAvPAL-PEG in cynomolgus monkey and human serum
BAS-VR-06-003	Detection of IgM antibodies to PEGylated *Anabaena variabilis* Phenylalanine Ammonia Lyase (rAvPAL-PEG) in rat, monkey and human serum
BAS-VR-06-005	Detection of PEGylated Recombinant *Anabaena variabilis* Phenylalanine Ammonia Lyase (rAvPAL-PEG) in rat, monkey and human serum
BAS-VR-07-001	Detection of IgG antibodies to PEGylated *Anabaena variabilis* Phenylalanine Ammonia Lyase (rAvPAL-PEG) in monkey and human serum
BAS-VR-07-004	Validation of Phenylase total protein by UV Spectrophotometry
BAS-VR-07-005	Dose Concentration Analysis of PEGylated Recombinant *Anabaena variabilis* Phenylalanine Ammonia Lyase (rAvPAL-PEG) in Formulation Buffer
BAS-VR-11-005	Quantitative Measurement of PEGylated Phenylalanine Ammonia Lyase (rAvPAL-PEG, BMN165) in Rabbit Plasma by ELISA
BAS-VR-11-007	Detection of Anti-rAvPAL IgG in Rabbit Serum
BAS-VR-12-026	Detection of anti-rAvPAL IgG antibodies in rat serum
BAS165-12-045	Detection of BMN165 in Rabbit Plasma using a Ligand Binding Assay

BMN165-12-046	Detection of BMN165 in Rat Plasma using a Ligand Binding Assay
BMN165-13-068	Development and Validation of an Immunohistochemical Method for the Semi-Quantitative Evaluation of Anti-PEG Antibody and Anti-rAvPAL Antibody Binding in Formalin-Fixed Paraffin-Embedded Rat Tissues
BMN165-14-069	Quantitation of BMN165 in Rat Breast Milk by Electrochemiluminescence
ARPHE1	Monkey Plasma Phe LC/MS/MS Assay
ARPHE2	Rabbit Plasma Phe LC/MS/MS Assay
ARPHE3	Rat Plasma Phe LC/MS/MS Assay
16-RS-357-VAL	Validation of an Anti-Polyethylene Glycol (PEG) Immunohistochemistry Assay in Preserved Rat Tissues
McCaman, et. al.	Fluorometric method for the determination of phenylalanine in serum (Fluorimetric method for the determination of phenylalanine in serum. J Lab Clin Med 1962 (59): 885-890)

3.3 Previous Reviews Referenced

N/A

4 Pharmacology

4.1 Primary Pharmacology

Dose Range Reduction Study of rAvPAL-PEG for 16 Weeks in ENU2 Mice with a 2 Week Recovery Period (Study #: 0165-07-001)

Method:

Homozygous ENU2 mice (also known as BTBR*Pah*enu2) (8/sex/group) were administered rAvPAL-PEG at doses of 0 (vehicle), 20, 40, or 80 mg/kg weekly via subcutaneous (SC) injection for a total of 16 weeks. Mice in each dose group were injected weekly at the highest dose level for 10 weeks, and then were continued at a reduced dose level for additional 6 weeks. The vehicle was 10 mM Tris with 140 mM NaCl (pH 7.5). The dose volume was 10 mL/kg. Animal ages at initial dose administration ranged from 3 to 5 months. Following the 16th dose on day 106, animals were monitored during a 2-week recovery period. The Sponsor's table below summarizes the treatment groups for this experiment.

Table 2: Group Designations and Dose Levels

Group #	Animal Numbers	Administered	Weeks	Dose Volume (mL/kg)	Test Article Concentration (mg/mL)	Nominal Dose Level (mg/kg)	Nominal Activity Levels (U/mouse)
1	1-16	Vehicle/PEG	1-10	10	0	0	0
			11-16	10	0	0	0
2	17-32	rAvPAL-PEG	1-10	10	8	80	4
			11-16	10	4	40	2
3	33-48	rAvPAL-PEG	1-10	10	8	80	4
			11-16	10	2	20	1
4	49-64	rAvPAL-PEG	1-10	10	4	40	2
			11-16	10	2	20	1

Clinical signs (morbidity, mortality and general health) and coat color were observed daily. Body weight was recorded weekly. Blood samples for analysis of Phe levels or anti-drug antibodies were collected at 1 day prior to first dose and on study days 5, 8, 12, 15, 19, 22, 26, 29, 33, 36, 40, 43, 47, 50, 54, 57, 61, 64, 68, 71, 75, 78, 82, 85, 89, 92, 96, 99, 103, 106, 110, 113, 117 and 120 (prior to dosing where applicable).

Plasma phenylalanine (Phe) analysis was conducted using a 96-well plate fluorescence assay adapted from the protocol of Sigma Phenylalanine Diagnostic kit 60 (discontinued) by the BioMarin Analytical Chemistry laboratory.

Phenylalanine ammonia lyase (PAL)-specific IgG and IgM antibody levels in sera were measured by an ELISA assay using PAL as the capturing antigen in the BioMarin BioAnalytical Research and Development (BARD) laboratory. Anti-PAL IgG was detected with biotinylated goat anti-mouse IgG antibody and Streptavidin-HRP conjugates.

Results:

1. Mortality, clinical signs and body weights

There were four unscheduled deaths, which included one each in groups 1 (vehicle control) and 4 (40 mg/kg → 20 mg/kg) and two in group 2 (80 mg/kg → 40 mg/kg). The group 1 control female was sacrificed on day 47 after 7 doses due to lethargy, hunching and lack of movement. A male in group 4 was found dead on day 70 after 10 doses.

This animal had no clinical signs prior to death. Two females in group 2 were sacrificed on days 85 or 119 due to moribund state, hypothermia, and lethargy. No necropsy was performed on these animals.

Impairment of melanin biosynthesis due to hyperphenylalaninemia was reversed by rAvPAL-PEG treatment, as evidenced by the growth of new bands of black hair on the body and face following treatment. Change of coat color was observed in animals receiving 80 mg/kg or 40 mg/kg on days 11 and 12, respectively. Animals in groups 2 and 3 had coat color change by days 29 and 32, respectively. All animals in the treatment groups had darkened coat color by day 49 of treatment.

rAvPAL-PEG had no significant effects on body weight.

2. Plasma Phe levels

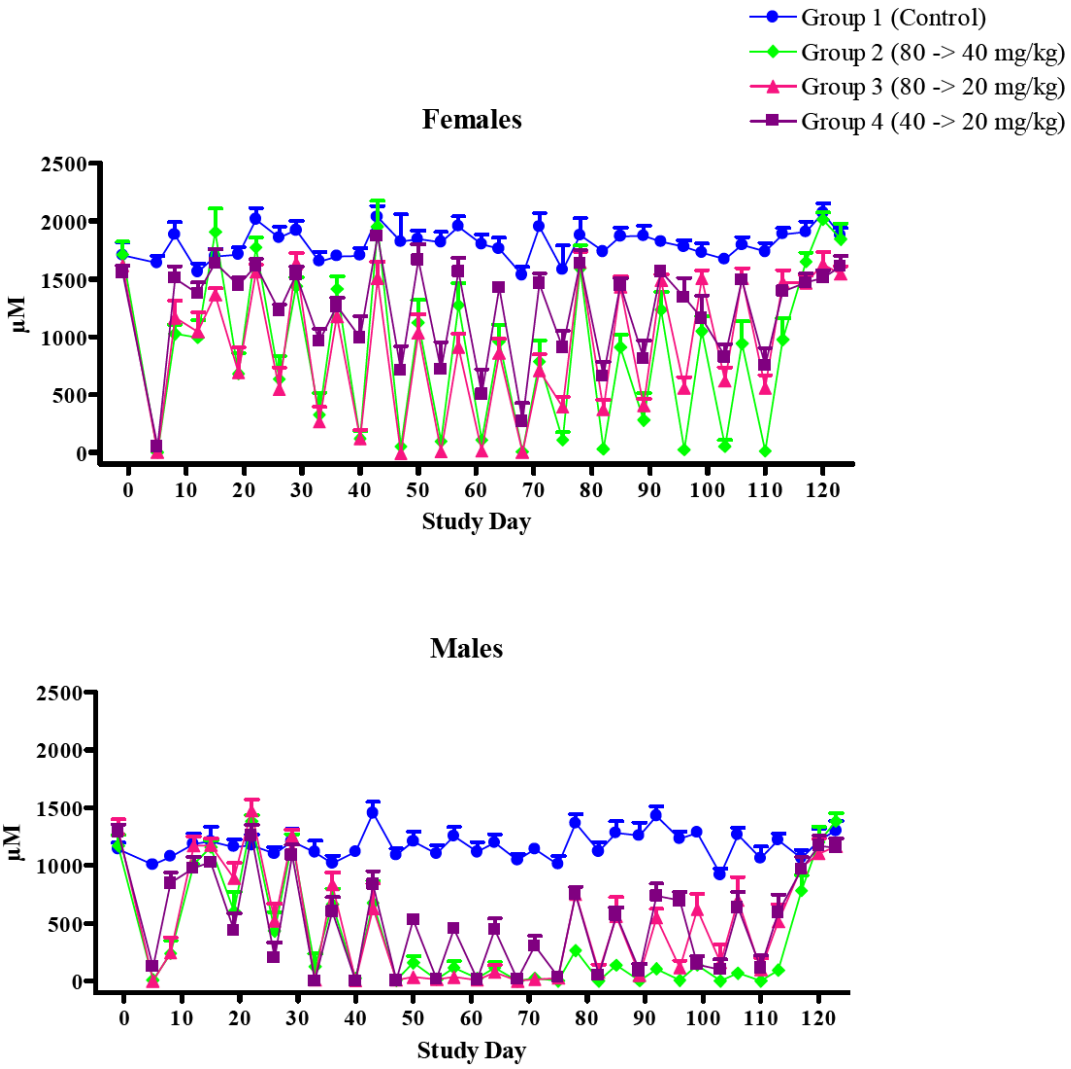
Plasma Phe was reduced in animals administered 80 or 40 mg/kg by day 4 after the first injection. However, an attenuated response in reducing plasma Phe concentrations was observed in all treated males during weeks 3 through 7, and in all treated females during weeks 3 through 16.

Male ENU2 mice receiving 80 mg/kg rAvPAL-PEG had stable plasma Phe concentrations of less than 200 μM after 8 weeks of treatment. However, male mice that received 40 mg/kg for 8 weeks had plasma Phe concentrations that ranged up to ~500 μM throughout the week. Therefore, the dose regimen of weekly SC administrations of 80 mg/kg for ten weeks and then 40 mg/kg for six weeks was the most effective regimen in achieving stable plasma Phe levels over the entire week in male ENU2 mice.

Overall, females had higher baseline plasma Phe concentrations than males throughout the study. Weekly SC administration of 80 and 40 mg/kg rAvPAL-PEG reduced plasma Phe concentrations, but did not maintain plasma Phe concentrations over the entire week in female ENU2 mice.

The Phe levels were comparable to the control values after discontinuing treatment for two weeks. The plasma Phe levels are summarized in the figures below (taken from the study report).

Figure 1: Mean Plasma Phenylalanine Levels in ENU2 Mice Administered rAvPAL-PEG or Vehicle SC Weekly for 16 Weeks



High dose from weeks 1 to 10 (days 1 to 77), reduced dose from weeks 11 to 16 (days 78 to 113)

3. Serum anti-rAvPAL-PEG antibodies

Anti-rAvPAL-PEG IgG was detected on days 22 to 33 and thereafter in all treated ENU2 mice. The titers remained ≤12150 throughout the study period. There was no clear relationship between titer and dose levels.

In conclusion, rAvPAL-PEG at all doses decreased plasma Phe and produced darkening of coat color in ENU2 mice. The dose regimen of weekly SC administration of 80 mg/kg for ten weeks and then 40 mg/kg for six weeks was the most effective

regimen in achieving stable plasma Phe levels in male ENU2 mice. However, weekly SC administration of rAvPAL-PEG at all doses was not as effective in stabilizing plasma Phe levels in female ENU2 mice. The reduced dose of 20 mg/kg for the last 6 weeks of the study appeared ineffective in both sexes. Anti-rAvPAL-PEG IgG was detected in all treated animals without a clear relationship between titers and dose levels.

Pharmacology Study Evaluating Plasma Phenylalanine Levels in ENU2 Mice Following Subcutaneous Administration of AvPAL and PEGylated AvPAL (Study #: 0164-06-019)

Methods:

The objective of this study was to evaluate the ability of wtAvPAL (recombinant wild-type PAL from *Anabaena variabilis*) and PEGylated AvPAL (wtAvPAL-PEG) to reduce plasma phenylalanine (Phe). PEGylated R91K (R91K-PEG) PAL was a recombinant PAL from *Rhodosporidium toruloides* containing the mutation R19K *toruloides* to which methoxypolyethylene glycol (PEG) is attached. PEGylated R91K PAL was used in this study as a positive control.

Both male and female ENU2 mice were administered AvPAL, wtAvPAL-PEG, or R91K-PEG via SC injection on days 1, 4, or 8. The study design is summarized in the Sponsor's table below.

Table 3: Group Designations and Dose Levels

Group	Compound	Dose Level (IU/mouse)[a]	Dose (mg/ mouse)	Dose Regimen	No. and Gender per Group	
					Males	Females
1	Vehicle	0	0	Days 1, 4, 8	3	0
2	wt AvPAL[b]	0.2	0.11	Days 1, 4, 8	1	4
3	wt AvPAL[b]	1.0	0.53	Days 1, 4, 8	5	0
4	wt AvPAL PEG[c]	0.2	0.13	Days 1, 4, 8	3	2
5	wt AvPAL PEG[c]	1.0	0.66	Days 1, 4, 8	2	4
6	R91K PEG[d]	1.0	0.59	Days 1, 4, 8	3	2

Study design includes evaluation PEGylated and non-PEGylated AvPAL administered SC to ENU2 mice.

[a] Groups 1-6 were dosed at a volume of 0.33 mL/mouse.

[b] Lot # 0164-03262006-00Av.

[c] Lot # 0164-03262006-00Av-X2.

[d] Lot # 0164-03062006-01-X2.

Morbidity, mortality and general health were observed daily. Body weights were recorded weekly.

Blood collections were performed 3 days prior to first dose (-3) and prior to dosing on study days 2, 4, 5, 8, 9, 12 and 22 via tail vein bleed. On days -3, 2, 4, 5, 8, 9 and 12,

plasma was collected for Phe analysis. On days -3, 8 and 22, serum was collected for anti-PAL IgG titer analysis.

Plasma Phe analysis was conducted using a 96-well plate fluorescence assay adapted from the protocol of Sigma Phenylalanine Diagnostic kit 60 (discontinued) by the BioMarin Analytical Chemistry laboratory.

PAL-specific IgG antibody levels in sera were measured by ELISA assay using PAL as the capturing antigen by the BioMarin BioAnalytical Research and Development (BARD) laboratory.

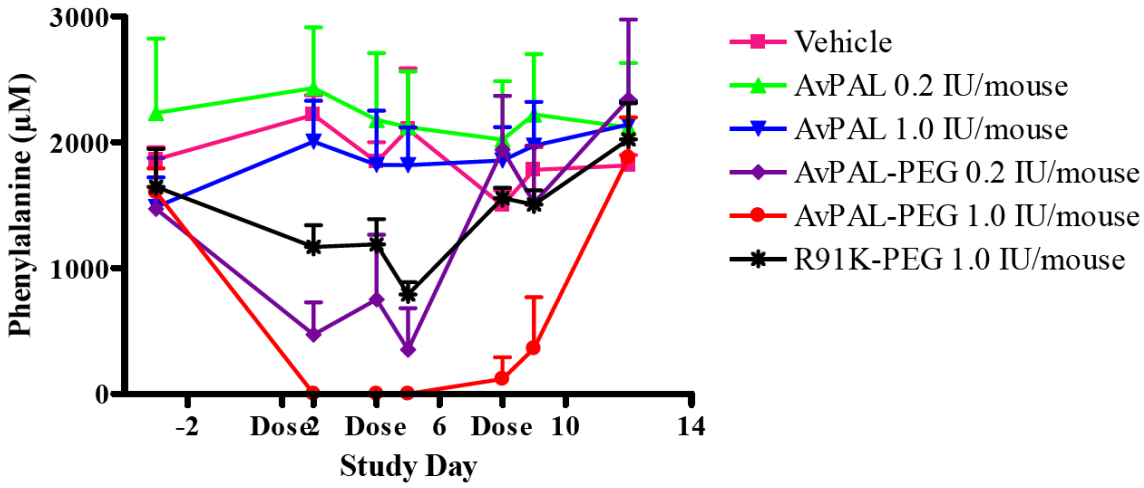
Results:

There were no mortalities. There were no treatment-related changes in clinical signs, morbidity or body weight. One male in group 3 was removed from the study on day 1 due to insufficient dose solution volume. One female in group 4 (# 4) and one male in group 3 (# 13) each received 2/3 of their scheduled dose on day 1 due to insufficient dose solution volume.

SC administration of multiple doses of 0.2 and 1 IU wtAvPAL/mouse did not significantly change plasma Phe in ENU2 mice over 12 days (see figure below).

As shown in the figure below (taken from the study report), wtAvPAL-PEG at doses of 0.2 or 1 IU/mouse and PEGylated R91K PAL at 1 IU/mouse reduced plasma Phe concentrations in ENU2 mice after the first and second dose on study days 1 and 4, respectively. However, the PD effects of PEGylated R91K PAL were at least 5-times less compared to that of the wtAvPAL-PEG at the same dose level. Following the third dose, wtAvPAL-PEG at 0.2 IU/mouse and PEGylated R91K PAL at 1 IU/mouse failed to reduce Phe concentration. A dose-dependent reduction in Phe level was observed for wtAvPAL-PEG.

Figure 2: Mean Plasma Phenylalanine levels in ENU2 mice administered PAL, SC on study days 1, 4 and 8



Anti-PAL IgG was first detected on day 8, and the titers increased as treatment duration increased. wtAvPAL induced a moderate to high immune response by study day 22 (IgG antibody titers: 109,035 - ≥ 328,050) while wtAvPAL-PEG induced a low immune response (IgG antibody titers: < 50 to 36,450). R91K PEG-PAL (IgG antibody titers: 12,150 – 36,450) produced a mild immune response on day 22, compared to wtAvPAL.

The observed lost or attenuated PD effect of all AvPAL-PEG formulations were likely due to the generation of neutralizing antibodies in all treatment groups during the study period.

In conclusion, PEGylation of wtAvPAL is necessary to allow for the pharmacodynamic activity of AvPAL. The formation of anti-AvPAL IgG antibodies appears to neutralize the PD effect of wtAvPAL-PEG.

Repeat Dose Study of PEGylated R91K PAL, wtAvPAL, and NpPAL in ENU2 Mice (study report #: 0164-06-041, 0164-06-053, and 0164-06-060)

Methods:

The Sponsor submitted a combined final study report of the following three studies:

1). Seven Week Repeat Dose Study of PEGylated R91K PAL (phenylalanine ammonia lyase) (R91K-PEG), PEGylated wtAvPAL (wtAvPAL-PEG) and PEGylated NpPAL (NpPAL-PEG) in ENU2 Mice (Study 0164-06-041)

2). Continuation of Groups 1 and 2 from Study 0164-06-041: A 4-Week Repeat Dose Study of wtAvPAL-PEG and NpPAL-PEG in ENU2 Mice (Study 0164-06-053)

3). Continuation of Group 2 from Study 0164-06-053: Additional Eight Week Repeat Dose Study of wtAvPAL-PEG in ENU2 Mice Assessing Maintenance Doses (Study 0164-06-060)

RtPAL, AvPAL and NpPAL are variants of phenylalanine ammonia lyase (PAL) produced by *Rhodosporidium toruiedis, Anabaena variabilis* and *Nostoc punctiforme,* respectively. R91K is a mutant form of RtPAL.

The objective of these studies was to determine plasma phenylalanine (Phe) levels in male ENU2 mice following repeated weekly administration of equivalent subcutaneous administrations of different PEGylated phenylalanine ammonia lyase enzymes (PALs).

The initial study (# 0164-06-041) assessed the Phe-lowering ability of these three different PEGylated PAL variants. At the end of this initial study, animals administered NpPAL-PEG and AvPAL-PEG were transferred to a new study (# 0164-06-053) and continued to receive weekly administrations of PAL to further evaluate long-term effects. At the end of study 0164-06-053, animals receiving AvPAL-PEG continued to receive treatment under study 0164-06-060 to evaluate Phe-lowering ability of lower doses of AvPAL-PEG. The study design is summarized in the Sponsor's table below.

Table 4: Group Designations and Dose Levels

Study	Group #	N	Test Article	Dose level (IU/ dose)	Number of Doses	Dose Concentration (IU/mL)	Injection Volume (mL)
0164-06-041	1	4	NpPAL-PEG	1	7	3.3	0.30
	2	4	AvPAL-PEG	4	7	13.3	0.30
	3	4	R91K-PEG	6	7	20	0.30
	4	4	Tris NaCl Vehicle	0	7	0	0.30
Days between last dose of 0164-06-041 and first dose of 0164-06-053: 15							
0164-06-041	1	4	NpPAL-PEG	1	4	3.3	0.30
	2	3	AvPAL-PEG	4	4	13.3	0.30
Days between last dose of 0164-06-053 and first dose of 0164-06-060: 48							
0164-06-060	2	4	AvPAL-PEG	4	2	13.3	0.30
0164-06-060	2	4	AvPAL-PEG	2	2	13.3	0.15
0164-06-060	2	4	AvPAL-PEG	1	1	13.3	0.075

HC –high concentration
NOF –Nippon Oil and Fat
PAL –phenylalanine ammonia lyase
PEG – polyethylene glycol

Morbidity, mortality and general health were observed daily. Body weights were recorded twice per week in the first two weeks, and then weekly. Blood collections were performed pre-dose and during the treatment period.

Plasma phenylalanine (Phe) analysis was conducted using a fluorimetric assay modified for small volumes and 96-well microtiter plate format.

PAL-specific IgG and IgM antibody levels in sera were measured by ELISA assay using PAL as the capturing antigen by the BioMarin BioAnalytical Research and Development (BARD) laboratory.

Results:

1. Mortality and body weight

There was no treatment-related death. One animal in group 2 (# 7) died on day 22 during the blood collection. The death was attributable to the blood collection. There were no treatment-related changes in body weight.

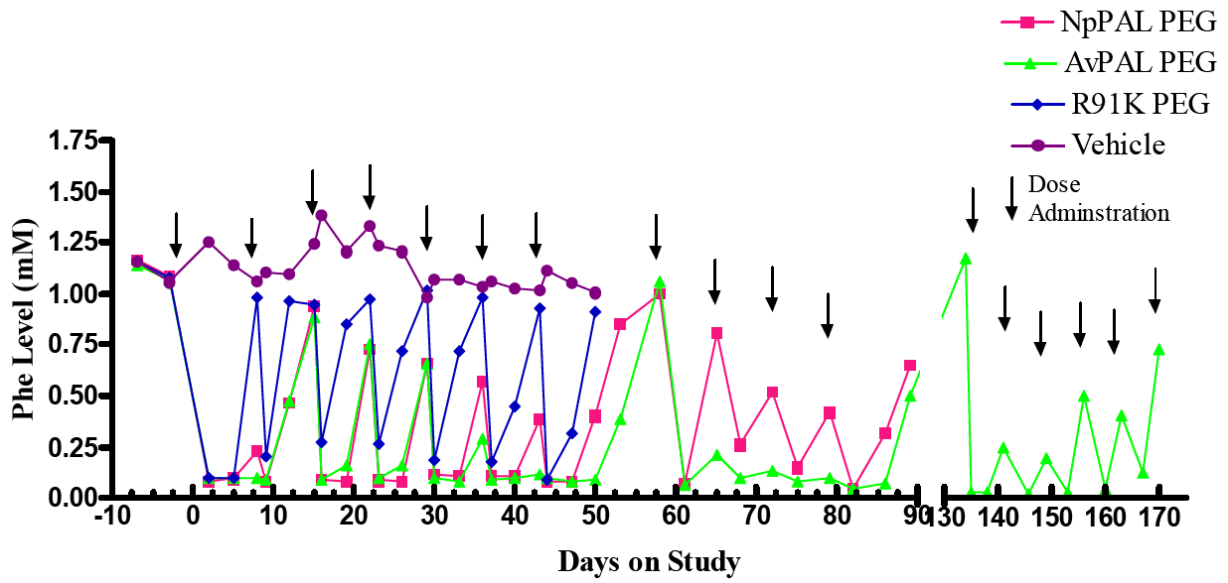
2. Plasma Phe levels

As shown in the Sponsor's figure below, overall, following each dose of the three variants (6 IU/dose R91K-PEG, 4 IU/dose wtAvPAL-PEG or 1 IU/dose NpPAL-PEG) in ENU2 mice, plasma Phe levels were reduced below 0.2 mM.

After the first two doses, there was an attenuated Phe reduction in animals administered 6 IU R91K-PEG over weeks 3 through 6. Loss of pharmacodynamic effect was also observed in animals administered 1 IU/dose NpPAL-PEG and 4 IU/dose AvPAL-PEG, although the range of Phe levels over the week decreased with time. NpPAL-PEG and AvPAL-PEG maintained lower Phe levels than R91K-PEG throughout the entire week during the 7-week treatment period.

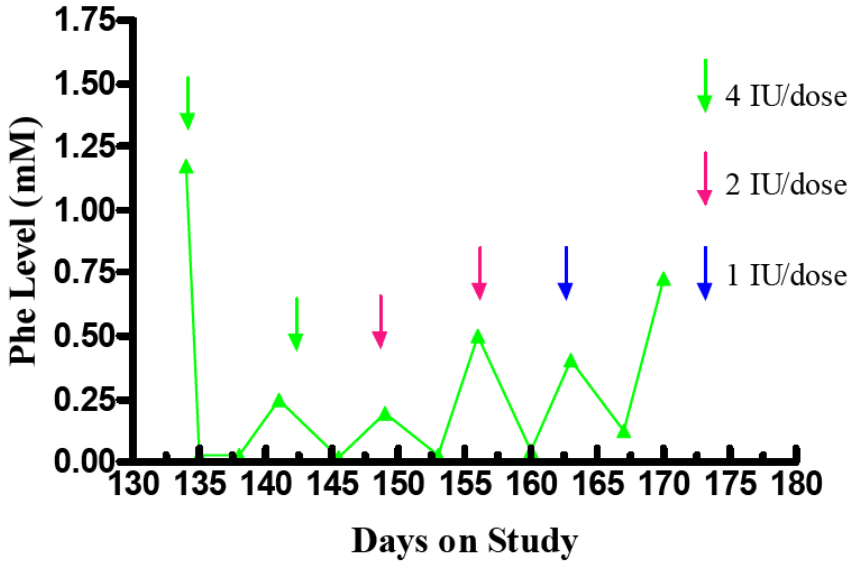
Weekly SC administration of 4 IU/dose AvPAL-PEG but not 1 IU/dose NpPAL-PEG (study 0164-06-060) maintained plasma Phe levels below 0.5 mM in ENU2 mice throughout the 4-week treatment period.

Figure 3: Group Mean Phe Levels in ENU2 Male Mice Administered Three PAL Variants via SC Injection Weekly



In a dose de-escalation study (study 0164-06-060), ENU2 mice in wtAvPAL-PEG treatment group were administered wtAvPAL-PEG at doses of 4 IU, 2 IU or 1 IU weekly for 2 weeks. A weekly SC dose of 4 IU, but not 2 IU or 1 IU wtAvPAL-PEG, maintained plasma Phe levels below 0.5 mM in ENU2 mice (see Sponsor's figure below).

Figure 4: Group Mean Phe Levels in ENU2 Male Mice Receiving wtAvPAL-PEG in a Dose De-escalation in Study 0164-06-060



3. Serum anti-PAL IgG antibody

NpPAL-PEG administration produced the strongest anti-PAL IgG antibody response with titers up to 109,350 at days 15, 29 and 50. wtAvPAL-PEG administration produced the lowest anti-PAL IgG antibody response with a high titer of 12,150 at days 29 and 50. SC administration of R91K-PEG resulted in a mild antigenic response with anti-PAL IgG titers of up to 36,450 on days 29 and 50. Extension of weekly SC administration of NpPAL-PEG or wtAvPAL-PEG resulted in a reduced anti-PAL IgG response.

In conclusion, R91K-PEG reduced plasma Phe, but did not have a lasting effect as Phe values were similar to those in the vehicle control group within 1 week of each dose. NpPAL-PEG and wtAvPAL-PEG each produced reductions in plasma Phe which were maintained with continued dosing. R91K-PEG and NpPAL-PEG treated animals developed anti-PAL antibodies with a high titer, while wtAvPAL-PEG produced a lower titer antigenic response.

Evaluation of Plasma Phenylalanine Levels Following Subcutaneous Administration of PEGylated AvPAL variant in Naïve ENU2 Mice (Study # 0165-06-057)

Methods:

The objective of this study was to determine plasma Phe concentrations following repeated SC administration of PEGylated wild-type AvPAL (AvPAL-PEG) or rAvPAL-PEG (amino acid substitutions C503S and C565S) at multiple dose levels in ENU2 mice.

Male and female ENU2 mice (2 - 4 months old; 1/sex for control group, and 2/sex/for treatment group) were treated with weekly SC administration of rAvPAL-PEG at doses of 0.25, 1, or 4 IU/mouse for 8 weeks. The dose volume was 0.33 mL. Wild-type AvPAL-PEG (AvPAL-PEG) at a dose of 4 IU/mouse was also administered as a comparator. The vehicle was Tris NaCl (no concentration or pH were indicated). The study design is summarized in the Sponsor's table below.

Table 5: Study Design for Evaluation of Phenylalanine in ENU2 Mice

Group	Compound	Dose (IU/mouse)[a]	Nominal Dose (mg/mouse)	Dosing Frequency / Number	No. and Gender per Group	
					Male	Female
1	Vehicle	0	0	weekly / 8	1	1
2	rAvPAL-PEG[b]	0.25	0.23	weekly / 8	2	2
3	rAvPAL-PEG[b]	1.0	0.92	weekly / 8	2	2
4	rAvPAL-PEG[b]	4.0	3.67	weekly / 8	2	2
5	AvPAL-PEG[c]	4.0	3.81	weekly / 8	2	2

Study design includes evaluation of plasma phenylalanine concentrations after 8 weekly SC injections of wt AvPAL-PEG or rAvPAL-PEG in ENU2 mice.

[a] Groups 1–5 were dosed once per week at a volume of 0.33 mL/mouse.

[b] Lot # 0164-10122006-02Av-X2.

[c] Lot # 0164-10122006-02Av-X2.

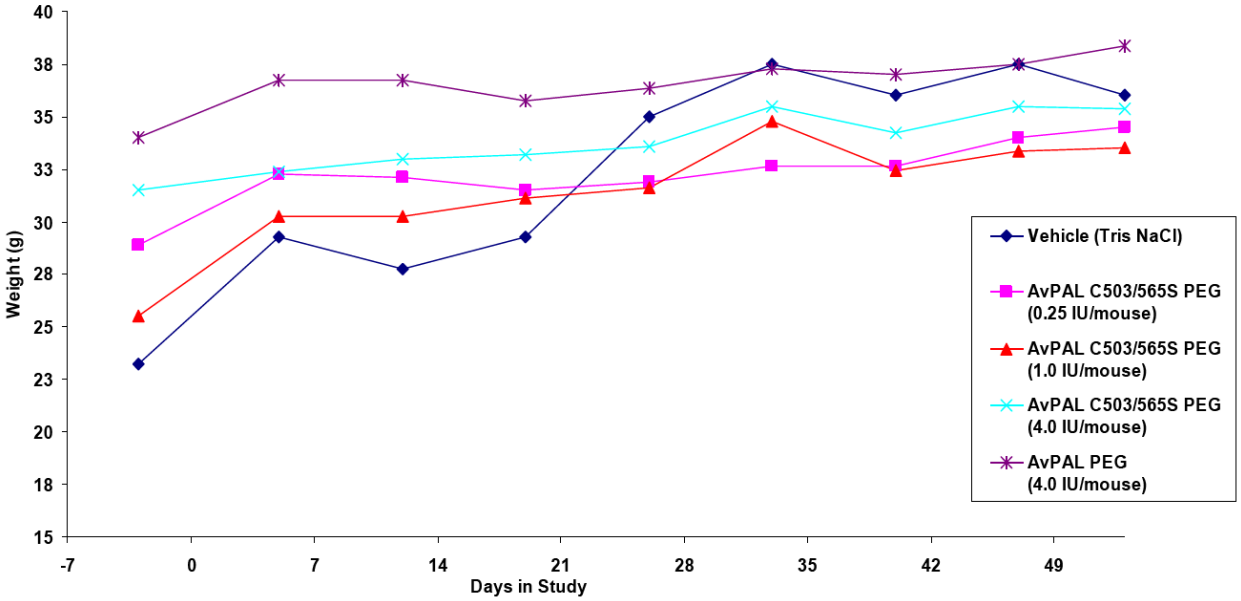
A pre-study blood collection was performed once at 3 days prior to first dose, followed by additional blood collections on study days 3, 8, 10, 15, 17, 22, 24, 29, 31, 36, 38, 43, 45, 50, 52, and 57 (prior to dose administration) for plasma Phe analysis. Serum was also collected at 3 days prior to the first dose, and on days 8, 15, 22, 29, 36, 43, 50, and 57 for anti-PAL IgG and IgM antibody analysis. Body weights were recorded prior to study initiation and weekly thereafter.

Plasma Phe levels were analyzed using a 96-well plate fluorescence assay adapted from the protocol of Sigma Phenylalanine Diagnostic kit 60 (discontinued) in the BioMarin Analytical Chemistry laboratory. PAL-specific IgG and IgM antibody levels were analyzed by the BioMarin BioAnalytical Research and Development (BARD) laboratory.

Results:

One male in the control group was sacrificed on day 25 due to an untreatable lesion. One female (#5) in group 2 was found dead on day 26. The Sponsor stated that neither death was considered to be treatment-related. All animals gained weight during the course of the study. Only one mouse (female) was in the control group by the end of the study period. Thus, it is not clear whether there was a treatment-related change in body weight (see Sponsor's figure below).

Figure 5: Group Mean Body Weights in ENU2 Mice Administered PAL via Weekly SC Injection for 8 Weeks



Plasma Phe levels:

The plasma Phe levels in ENU2 mice treated with rAvPAL-PEG and AvPAL-PEG via weekly SC injection are summarized in the Sponsor's figure below.

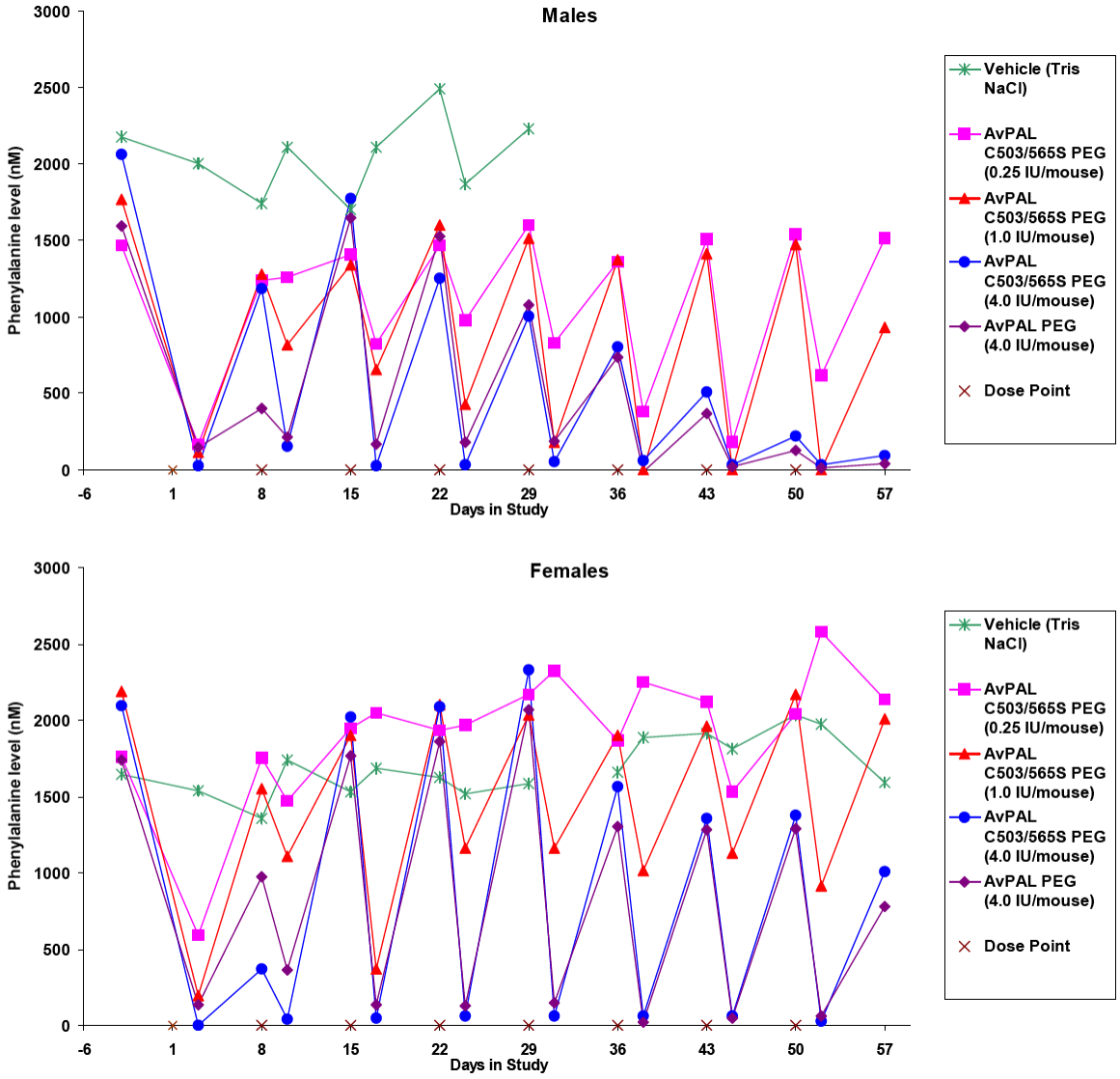
Each of the PEGylated PALs reduced plasma Phe levels following a single injection. The effectiveness of Phe reduction after weekly administration of rAvPAL-PEG over eight weeks appears to be dose-dependent. The rAvPAL-PEG at 4 IU/mouse was the most effective dose compared to the doses of 0.25 or 1 IU/mouse.

In male ENU2 mice, rAvPAL-PEG at all doses reduced plasma Phe levels to less than 500 nM after the first SC injection. Attenuated response was observed at all doses during weeks 2 through 7. Continuous low levels of plasma Phe (below 500 nM) were observed for one week after the eighth SC injection of 4 IU/mouse.

In female ENU2 mice, the changes in plasma Phe levels followed the same trends observed in the male ENU2 mice after weekly SC administration of rAvPAL-PEG. However, stabilization of plasma Phe levels over the entire week was not achieved over the 8-week treatment duration at all doses.

The ability to reduce plasma Phe levels by administration of 4 IU/mouse AvPAL-PEG was similar to that of rAvPAL-PEG (4 IU/mouse) during the 8-week treatment period.

Figure 6: Group Mean Plasma Phe Levels in ENU2 Mice Administered PAL via Weekly SC Injection for 8 Weeks



Serum anti-PAL antibodies:

There was no IgM data in the study report, and no rationale was provided for this omission. Anti-PAL IgG antibody was first detected in 5 of 16 treated ENU2 mice on day 8. On day 50, all treated mice except for one male in group 2 developed anti-PAL IgG antibodies. The titers of anti-PAL IgG did not appear to be dose- or treatment duration-dependent. One male in group 2 was negative for anti-PAL IgG antibody during the entire study period. Overall, animals treated with 4 IU rAvPAL-PEG had low titers of anti-PAL IgG compared to animals treated with 0.25 or 1 IU rAvPAL-PEG. rAvPAL-PEG treatment generated a lower average titer of anti-PAL IgG than the wild-type AvPAL-PEG. The anti-PAL titers in ENU2 mice treated with rAvPAL-PEG and AvPAL-PEG are summarized in the table below (taken from the study report).

Table 6: Anti-PAL IgG Titers in ENU2 mice administered PAL via Weekly SC Injection for 8 Weeks

Dose Group	Animal Number	Pre	Day 8	Day 15	Day 22	Day 29	Day 36	Day 43	Day 50	Day 57
Vehicle (Tris NaCl)	1	<50	<50	<50	<50	<50	<50	N/A*	<50	<50
	2	50	<50	50	<50	N/A*	N/A*	N/A*	N/A*	N/A*
AvPAL C503/565S PEG 0.25 IU/mouse s.i.w.	3	<50	50	<50	50	50	<50	150	150	<50
	4	<50	50	50	50	450	>1350	>1350	>1350	4050
	5	<50	<50	<50	<50	<50	N/A*	N/A*	N/A*	N/A*
	6	<50	<50	50	50	50	<50	50	50	<50
AvPAL C503/565S PEG 1.0 IU/mouse s.i.w.	7	<50	<50	<50	<50	<50	50	150	>1350	150
	8	<50	<50	<50	<50	<50	<50	<50	<50	<50
	9	<50	<50	<50	<50	50	450	>1350	>1350	450
	10	<50	<50	<50	<50	<50	<50	50	50	<50
AvPAL C503/565S PEG 4.0 IU/mouse s.i.w.	11	<50	<50	<50	50	50	50	150	50	50
	12	<50	<50	50	50	50	150	150	150	50
	13	50	50	50	450	150	450	450	450	150
	14	<50	<50	<50	<50	150	150	150	150	150
AvPAL PEG 4.0 IU/mouse s.i.w.	15	<50	<50	150	150	450	450	1350	1350	150
	16	<50	50	150	150	450	150	450	450	150
	17	<50	<50	150	4050	12150	4050	4050	4050	450
	18	<50	50	150	450	150	50	50	50	<50

* no sample/data not available.

In conclusion, weekly SC administration of rAvPAL-PEG at doses of 0.25, 1, or 4 IU/mouse produced a dose-dependent reduction in plasma Phe in ENU2 mice. An attenuated response in the ability to reduce plasma Phe concentrations was observed between weeks 2 through 7. The ability to reduce Phe concentrations appears to be approximately equivalent between rAvPAL-PEG and the wild-type AvPAL-PEG. Anti-PAL IgG antibodies developed in almost every mouse in the treatment groups. The rAvPAL-PEG generated a lower average titer of anti-PAL IgG than the wild-type AvPAL-PEG at the same dose level.

Evaluation of Plasma Phenylalanine Levels Following Subcutaneous Administration of PEGylated AvPAL variant in Naïve ENU2 Mice (Study#: 0165-06-058)

Methods:

This study evaluated the plasma Phe-lowering activity of AvPAL C503S/C565S (rAvPAL) PEGylated at different ratios with 20 KDa linear PEG in naïve male ENU2 mice. This study included two periods.

In the first study period, male ENU2 mice (4-6 months old) were given weekly SC administration of AvPAL-PEG or rAvPAL-PEG at a dose of 4 IU/mouse for 8 weeks. The dose volume was 0.33 mL. rAvPAL was PEGylated at ratios of 1:1.6, 1:2.4, or 1:3 (rAvPAL: 20 KDa linear PEG). The vehicle was Tris NaCl (concentration and pH were not indicated). The study design is summarized in the table below (taken from the study report).

Table 7: Group Designations and Dose Levels for the First Study Period (Weeks 1-8)

Group #	N	Administered	Dose Level (IU/mouse)	Dose Level (IU/mouse)	Dose Volume (mL)	Total Number of Doses and Dose Frequency
1	4	AvPAL PEG (1:3 ratio)	4	3.81	0.33	8, s.i.w. (once weekly administration, on day 1 of a 7 day schedule)
2	4	AvPAL C503S/C565S PEG (1:1.6 ratio)	4	2.84	0.33	
3	4	AvPAL C503S/C565S PEG (1:2.4 ratio)	4	3.03	0.33	
4	4	AvPAL C503S/C565S PEG (1:3 ratio)	4	3.67	0.33	
5	2	Vehicle	0	0	0.33	

Blood collection was performed once at 5 days prior to first dose and on study days 2, 5, 8, 12, 15, 19, 22, 26, 29, 33, 36, 40, 43, 47, 50, 54, 57, and 61 (prior to dosing where applicable) for plasma Phe analysis. Serum was also collected 2 days prior to the first dose and on days 15, 28, 43, and 61 for PAL-specific IgG and IgM antibody analysis. Body weights were recorded prior to study start and weekly thereafter.

After a 78-day treatment-free period, mice in groups 1 (AvPAL-PEG 1:3, n = 4), 4, (rAvPAL-PEG 1:3, n = 4), and 5 (control group, n = 1) were enrolled in a 16-week study extension. Eight mice were administered rAvPAL-PEG (1:3) at a dose of 4.3 IU/mouse via weekly SC injection for a total of 15 doses. The control group was administered saline (0.9% NaCl). The study design is summarized in the Sponsor's table below.

Table 8: Group Designations and Dose Levels after a 78-day Hiatus in a 16-Week Extension Study

Group #	N	Administered	Dose Level (IU/mouse)	Dose Level (IU/mouse)	Test Article Concentration (mg/mL)	Dose Volume (mL)	Total Number of Doses and Dose Frequency
1 and 4	8	AvPAL C503S/C565S PEG (1:3 ratio)	4.3	2.64	8	0.33	15, s.i.w. (once weekly administration, on day 1 of a 7 day schedule**)
5	1	NaCL		0	0	0.33	15, s.i.w. (once weekly administration, on day 1 of a 7 day schedule**)

** First dose was 1 day late, ie, there were 6 days between the first and second doses, following which the dosing was on day 1 of a 7 day schedule.

Blood collection was performed once at 1 day prior to first dose and on study days 2, 7, 10, 14, 17, 21, 24, 28, 31, 35, 38, 42, 45, 49, 52, 56, 59, and 63 (prior to dosing where applicable) for plasma Phe analysis. Serum was also collected pre-study and on days 18, 39, and 56 for PAL-specific IgG and IgM antibody level analysis. Body weights were recorded prior to study start and weekly thereafter.

Serum PAL-specific antibody levels were measured by the BioMarin BARD department using an ELISA assay with PAL as the capturing antigen. Anti-PAL IgG was detected with biotinylated goat anti-mouse IgG antibody and Streptavidin-HRP conjugates.

Plasma phenylalanine was measured using a fluorimetric assay modified for small volumes and a 96-well microtiter plate format.

Results:

One animal from the vehicle group died on day 57 at an age of approximately 7 months. The Sponsor attributed the death to reduced survivability of the ENU2 mouse line.

Two mice from the group administered rAvPAL-PEG (# 14, and 17) died during the 16 week extension study between study days 210 – 217. The Sponsor did not indicate the study days that the mice were found dead.

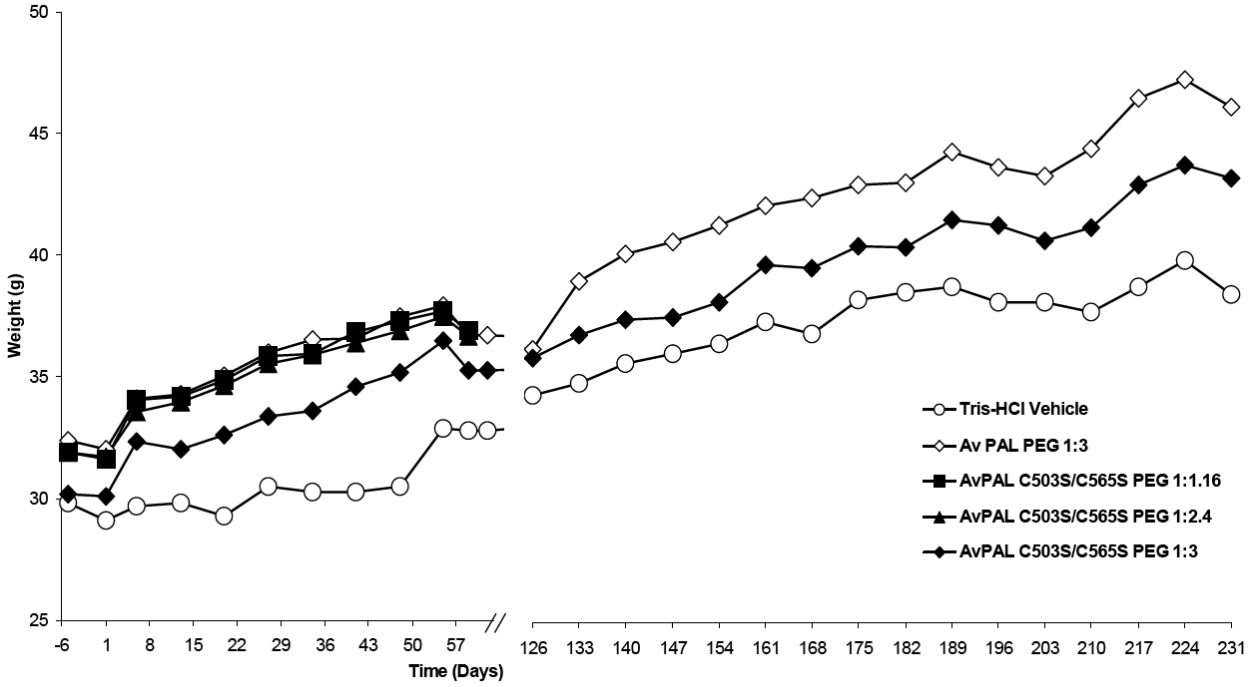
Hemangioma in urinary bladder, spermatic granuloma in bulbourethral gland, and hepatocellular carcinoma in the liver were observed in animal # 14. The Sponsor stated the following regarding this death: "*The lesions in the urinary and reproductive tract*

were non-lethal incidental findings. The cause of death of this animal was most likely liver failure secondary to hepatocellular carcinoma and was probably not test-article related."

An intestinal adenocarcinoma was found in animal # 17. The Sponsor stated the following about this finding: "*It cannot be determined whether this was test-article related. As this mouse died over the weekend, necropsy was not performed until Monday, and autolysis precluded histologic evaluation of tissues taken from this mouse.*"

All animals gained weight during the course of the study, but statistical analysis was not performed. The mean body weights are shown in the Sponsor's figure below.

Figure 7: Group Mean Body Weights in ENU2 Mice Administered PAL via Weekly SC Injection for 8 Weeks Plus 16 Week Extension

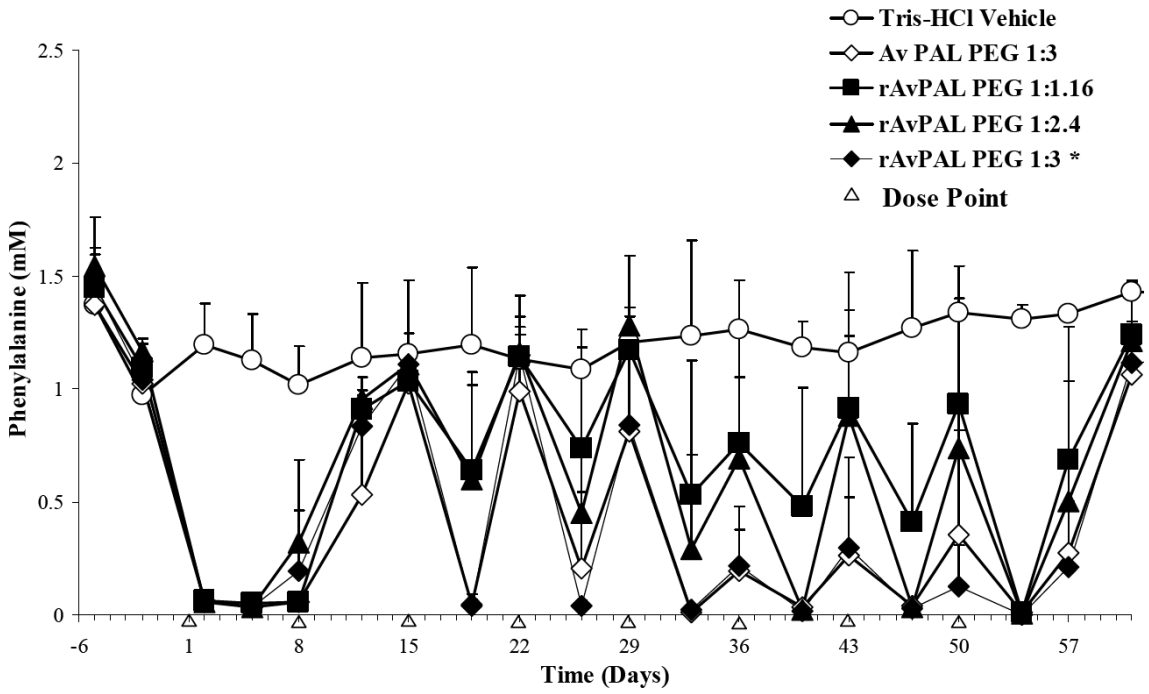


Plasma Phe levels

Each form of PEGylated rAvPAL reduced plasma Phe levels to less than 500 mM in ENU2 male mice following the first two injections. An attenuated ability to maintain reduced plasma Phe concentrations was observed during weeks 2 through 8. The effectiveness of plasma Phe reduction was directly proportional to the extent of PEGylation of rAvPAL-PEG. Following 6 weekly administrations, 1:3 PEGylated wtAvPAL-PEG and rAvPAL-PEG produced less fluctuation of plasma Phe concentration during the interval of SC administrations compared with the less PEGylated variants.

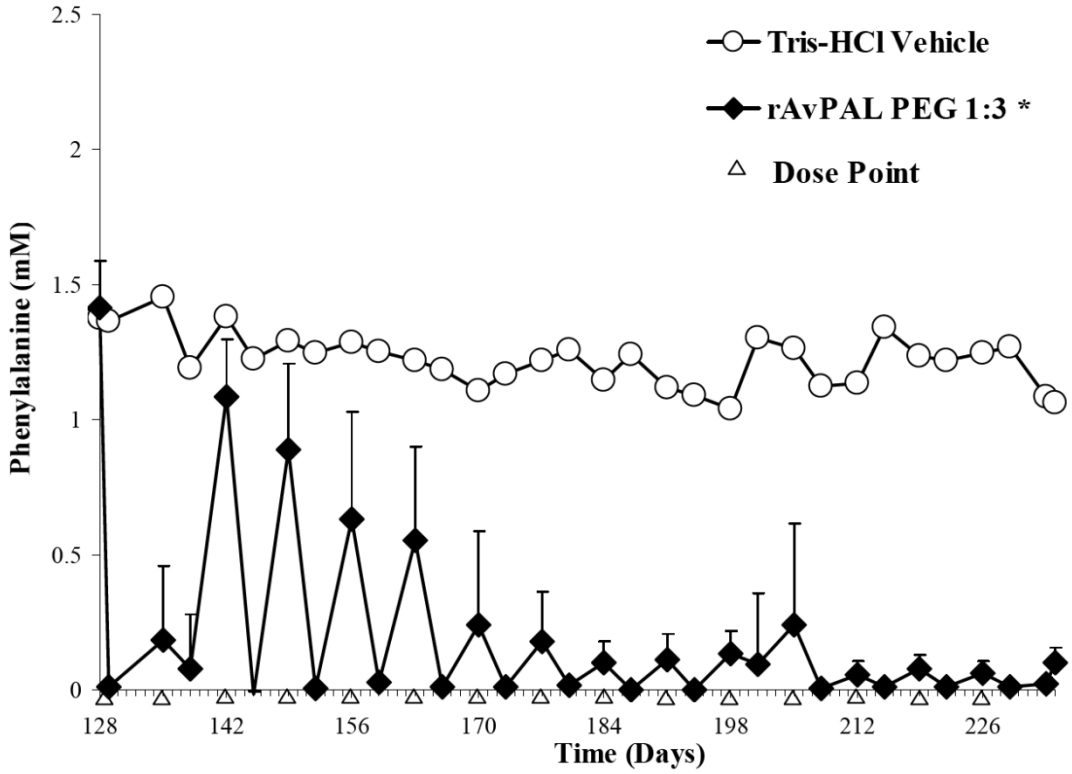
Thus, a higher degree of PEGylation of AvPAL resulted in a more stable reduction in plasma Phe. rAvPAL-PEG and wtAvPAL-PEG resulted in a similar Phe reduction over the 8 weeks in male ENU2 mice. The effects of differentially PEGylated rAvPAL-PEG on plasma Phe concentrations are shown in the figure below (taken from the study report).

Figure 8: Group Mean Plasma Phe Levels in ENU2 Mice Administered PAL via SC Weekly Injection for 8 Weeks



After 78-day hiatus, interim plasma Phe concentrations in ENU2 mice receiving rAvPAL-PEG showed an attenuated response from weeks 2 through 7, similar to that seen in the initial administration regimen (the 8-week study). A stable reduction in plasma Phe concentrations over the entire week was achieved after 7 weekly injections. Twelve weekly SC injections of rAvPAL-PEG resulted in stable plasma Phe concentrations below 0.2 mM. The mean plasma Phe levels in ENU2 mice treated with rAvPAL-PEG for 16 weeks are shown in the figure below (taken from the study report).

Figure 9: Group Mean Plasma Phe Levels in ENU2 Mice Administered rAvPAL-PEG via Weekly SC Injection for 16 Week Extension of Dosing



*Animals from group 1 (AvPAL PEG 1:3) were combined with group 4 (rAvPAL PEG 1:3) and dosed with rAvPAL PEG 1:3 beginning at Day 128

Serum anti-PAL antibodies

Anti-PAL IgM data was not included in the study report, and no rationale was given for this omission.

The rAvPAL with a ratio of 1:1.6 PEGylation produced the highest IgG titers. PEGylation at a 1:3 ratio produced a lower immunogenic response in both wild-type AvPAL and the rAvPAL, with a maximum titer of 1350. The antibodies were detected in most mice (14/16) on day 15 (the earliest blood collection time point). The anti-PAL IgG titers in ENU2 mice treated with PAL for 8-weeks are summarized in the table below (taken from study report).

Table 9: Anti-PAL IgG Titers in ENU2 Mice Administered PAL via SC Weekly Injection

Dose Group	Animal Number	Pre-dose	Day 15	Day 28	Day 43	Day 64
Av PAL PEG 1:3	1	<50	450	12150	4050	>1350
	6	<50	450	450	450	4050
	10	<50	50	50	150	450
	17	<50	150	450	450	1350
AvPAL C503S/C565S PEG 1:1.16	2	50	450	12150	1350	1350
	7	<50	1350	12150	12150	36450
	11	<50	450	1350	12150	12150
	18	50	150	36450	26.57M	>36450
AvPAL C503S/C565S PEG 1:2.4	3	<50	50	150	450	4050
	8	<50	50	50	50	450
	12	<50	50	150	450	4050
	13	<50	50	450	1350	4050
AvPAL C503S/C565S PEG 1:3	4	<50	50	50	450	450
	9	50	50	50	150	450
	14	<50	<50	50	450	1350
	16	<50	<50	150	50	450
Tris-HCl Vehicle	5	<50	<50	<50	<50	NA
	15	<50	<50	<50	<50	<50

NA = No sample (animal died prior to sample time-points)
M: The Sponsor did not indicate the meaning of M.

In conclusion, weekly SC administration of AvPAL-PEG or rAvPAL-PEG reduced plasma Phe in ENU2 mice. An attenuated ability to reduce plasma Phe concentrations was observed from weeks 2 through 8. The PD activity (plasma Phe reduction) was directly proportional to the extent of PEGylation of rAvPAL-PEG. A higher degree of PEGylation of AvPAL resulted in a more stable reduction in plasma Phe. In the 16-week extension study, ENU2 mice receiving rAvPAL-PEG (1:3) also showed an attenuated Phe concentration reduction from weeks 2 through 7. A stable reduction in plasma Phe concentrations over the entire week was achieved after 7 weekly injections. Twelve weekly SC injections of rAvPAL-PEG resulted in stable plasma Phe concentrations below 0.2 mM. A higher degree of PEGylation of AvPAL produced a lower antigenic response.

Comparison of rAvPAL-PEG and re-PEGylated rAvPAL-PEG in Naive Male ENU2 Mice (0165-07-032)	
Methods	**Results and Conclusion**
The objective of this study was to compare pharmacodynamics (PD) of rAvPAL-PEG and re-PEGylated rAvPAL-PEG. Re-PEGylated rAvPAL-PEG is PEGylated two times in separate, complete reactions with 20kDA of PEG at a ratio of 1:3. Male ENU2 (-/-) mice were treated with rAvPAL-PEG at doses of 80 mg/kg from week 1-8, then 20 mg/kg from week 9 to 12 via weekly SC injection, and followed by 3 weeks without treatment.	rAvPAL-PEG produced a dose-dependent decrease in plasma Phe with a corresponding increase in serum anti-rAvPAL IgG titers. Weekly treatment was not sufficient to maintain stable levels of Phe. Anti-rAvPAL-PEG IgG titer levels trended down and returned to approximately pre-dose levels by the final day of the post-treatment period. Data from the re-PEGylated rAvPAL-PEG treatment groups was not reported due to the absence of difference in PD markers (plasma Phe level and body weight) between the two lots.

Comparative Single-Dose Pharmacodynamics of Two rAvPAL-PEG Lots in Homozygote (-/-) Male BTBRPahenu2 Mice (0165-11-010)	
Methods	**Results and Conclusion**
The objective of this study was to evaluate the effects of rAvPAL-PEG derived from clipped or unclipped native enzyme on PD activity in male ENU2 mice. Male mice were treated with a single dose of 20 mg/kg rAvPAL-PEG derived from clipped or unclipped native enzyme via SC injection.	No difference in PD (reduction in plasma Phe levels) was observed between the two types of r-AvPAL-PEG. The use of clipped native rAvPAL enzyme to produce rAvPAL-PEG had no impact on the overall activity of rAvPAL-PEG.

Evaluation of Changes in Neuropathology Following Subcutaneous Administration of BMN 165 to Female BTBRPahenu2 Mice for 4, 8 or 12 Weeks (165-14-044)

Methods	Results and Conclusion
The objective of this study was to evaluate the effects of rAvPAL-PEG on neuropathological parameters in ENU2 mouse brain. Tyrosine hydroxylase (TH) or Nissl neurons in the hypothalamus and midbrain were evaluated by IHC in female ENU2 mice. Mice were treated with rAvPAL-PEG at doses of 10 (Monday), 10 (Wednesday), and 20 mg/kg (Friday) three times per week for 5, 8, or 12 weeks.	Partial reversal of the reduction in TH positive neurons in arcuate nucleus (ARC) of hypothalamus and dorsomedial hypothalamic nucleus (DMN) of midbrain in the ENU2 mice occurred at the three time points. rAvPAL-PEG increased the number of TH-expressing neurons in hypothalamus and midbrain, but the number was still lower compared to the values of wild type mice.

To identify a dose regimen that can stabilize Phe concentrations, support growth, and optimize reproductive capability in female ENU2 mice, the Sponsor conducted five studies (study #: 165-07-029, 165-08-005, 0165-08-027, 0165-09-068, 0165-10-008). Tested doses ranged from 5 to 120 mg/kg and were given via SC injection daily, weekly, twice weekly, or three times per week for a total of up to 50 weeks. The plasma Phe levels in treated mice were reduced to levels ranging from 0.1 to 600 µM, depending on the dose regimen. Plasma tyrosine levels were 40 - 50% lower compared to wild type mice when the Phe level was reduced to 200 µM, with no change in plasma tryptophan level. When Phe level was maintained between 200 to 600 µM by rAvPAL-PEG treatment, there was improvement in reproductive capacity as shown by the increased the rate of successful pregnancy (up to 52.6%) and increased pup survival (up to an average of 5.7 pups/litter).

However, when plasma Phe level was maintained between 0.1 to 0.2 µM by rAvPAL-PEG treatment, eight pregnant mice produced only two litters with 3 or 5 pups that survived until weaning. None of the tested dose regimens completely reversed the nearly complete litter loss due to maternal PKU syndrome in untreated ENU2 mice. Thus, the PD activity of rAvPAL-PEG in maintaining plasma Phe level in a narrow concentration range was likely the key factor in improvement of reproductive capacity in female ENU2 mice.

4.2 Secondary Pharmacology

N/A

4.3 Safety Pharmacology

Neurological Evaluation Study of rAvPAL-PEG in Rats (Study #: 0165-07-004)

Methods:

Sprague Dawley (SD) rats (5/sex/group) were administered a single dose of vehicle (10 mM Tris, 140 mM sodium chloride, pH 7.5) or 10, 50, or 125 mg/kg rAvPAL-PEG via subcutaneous (SC) injection. The dose volume was 5.95 mL/kg. After dosing, animals were observed for 5 days to assess the reversibility, persistence, or delayed occurrence of any effects. Animals were checked twice daily for mortality, abnormalities, and signs of pain or distress. Body weight was measured once during the pre-dose phase, on the dosing day and day of scheduled sacrifice. Prior to dose administration and at approximately 6, 24, 48 and 72 hours post-dose, each animal was subjected to a modified Irwin neurological assessment.

Results:

No treatment-related clinical signs were observed.

A significant decrease in body weight gain in males (45%, 14 g) and females (37%, 7 g) was observed at termination in the group treated with 125 mg/kg of rAvPAL-PEG, compared with control animals.

A lack of pupillary constriction to light stimuli was observed in male animals in the control group and across all treatment groups at 6 and 24 hr post-dose. The lack of pupillary constriction was not observed in control males at 48 and 72 hr post-dose, but was still observed in males in the treatment groups. Generally, a dose-dependent increase in incidence was observed. Mydriasis was observed in control males and in all male treatment groups at 6 hr post-dose. However, it persisted across all male treatment groups at 24 hr post-dose. No dose-dependent effect was observed. The incidence of lack of papillary constriction and mydriasis in males is summarized in the table below.

Table 10: Incidence of Lack of Pupillary Constriction and Mydriasis in Male SD rats Treated with rAvPAL-PEG

Signs		Male Groups (mg/kg)			
		0	10	50	125
6 hr post-dose					
Pupillary Status	Normal Eyes	2*	1	0	0
	Mydriasis, Eyes	3	4	5	5
Pupillary Response	Neither Pupil Constricts	3	4	4	5
	Both Pupil Constricts	2	1	1	0
24 hr post-dose					
Pupillary	Normal	5	4	0	3

Status	Eyes				
	Mydriasis, Eyes	0	1	5	2
Pupillary Response	Neither Pupil Constricts	2	4	5	5
	Both Pupil Constricts	3	1	0	0
48 hr post-dose					
Pupillary Status	Normal Eyes	5	5	5	5
Pupillary Response	Neither Pupil Constricts	0	0	2	3
	Both Pupil Constricts	5	5	3	2
72 hr post-dose					
Pupillary Status	Normal Eyes	5	5	5	5
Pupillary Response	Neither Pupil Constricts	0	1	1	3
	Both Pupil Constricts	5	4	4	2

*Number of animals with observed signs

A lack of pupillary constriction to light stimuli was observed in female animals in the control group and across all treatment groups at 6 hr post-dose. The lack of pupillary constriction was not observed in control females at 24 hr post-dose, but was still observed in treatment groups. At 48 hr post-dose, only one female in the 125 mg/kg group had this change. A dose-dependent effect was not observed. Mydriasis was observed in one control female and almost all treated females at 6 hr post-dose. However, this change was not observed at 24 hr and beyond. The incidence of lack of pupillary constriction and mydriasis in females is summarized in the table below.

Although lack of pupillary constriction to light stimuli and mydriasis were observed in control animals after dosing, animals in treatment groups had a higher incidence and the abnormal signs lasted a longer time after dosing. Thus, the lack of pupillary constriction to light stimuli and mydriasis may be treatment-related.

Table 11: Incidence of Lack of Pupillary Constriction and Mydriasis in Female SD rats Treated with rAvPAL-PEG

Signs		Female Groups (mg/kg)			
		0	10	50	125
6 hr post-dose					
Pupillary Status	Normal Eyes	5*	2	4	0
	Mydriasis, Eyes	0	3	1	5
Pupillary Response	Neither Pupil Constricts	1	5	4	5
	Both Pupil Constricts	4	0	1	0
24 hr post-dose					
Pupillary Status	Normal Eyes	5	5	5	5
	Mydriasis, Eyes	0	0	0	0
Pupillary Response	Neither Pupil Constricts	0	1	1	3
	Both Pupil Constricts	5	4	4	2
48 hr post-dose					
Pupillary Status	Normal Eyes	5	5	5	5
Pupillary Response	Neither Pupil Constricts	0	0	0	1
	Both Pupil Constricts	5	5	5	4
72 hr post-dose					
Pupillary Status	Normal Eyes	5	5	5	5
Pupillary Response	Neither Pupil Constricts	0	0	0	0
	Both Pupil Constricts	5	5	5	5

*Number of animals with observed signs

In conclusion, at a single SC dose of 125 mg/kg, rAvPAL-PEG significantly decreased male (↓45%) and female (↓37%) body weight gain. rAvPAL-PEG at all doses resulted in increased incidence and prolonged duration of absence of pupillary constriction to light

stimuli, and mydriasis. Thus, the lack of pupillary constriction to light stimuli and mydriasis of eyes may be treatment-related. rAvPAL-PEG had no effects on other parameters on the modified Irwin neurological assessment.

Effects of rAvPAL-PEG on Respiratory Functions in SD Rats Using the Head-Out Body Plethysmography Model (Study #: 0165-07-005)

Methods:

Sprague Dawley (SD) male rats in groups 1 through 4 (6/group) were administered a single dose of vehicle (10 mM Tris, 140 mM sodium chloride, pH 7.5) or 10, 50, or 125 mg/kg rAvPAL-PEG via SC injection. For the toxicokinetic study, male rats in groups 5 through 7 (3/group) were treated with the same dose regimen. The dose volume was 5.95 mL/kg. Respiratory function was assessed by measuring tidal volume, respiration rate, and minute volume using the head-out body plethysmography model. Head out plethysmography data from all animals in groups 1 to 5 was collected for approximately 90 min continuously. Animals were observed twice daily for mortality, abnormalities, or signs of pain or distress. Animals were weighed twice during the pre-dose phase, prior to dosing on the day of administration, and on the day of scheduled termination (groups 1-4 animals only in the main study groups). Plasma samples for TK analysis were collected at 6, 24, 48, and 72 hours post-dose. The study design is summarized in the table below (taken from the study report).

Table 12: Study Design in Respiratory Safety Pharmacology Study in Rats

Group	Compound[a]	Dose[b] (mg/kg)	Total Dose for Study (mg/kg)	Dosing Frequency	No. and Gender per Group	
					Main	TK
1	Vehicle	0	0	Once	6 M	
2, 5	rAvPAL–PEG	10	10	Once	6 M	3 M
3, 6	rAvPAL–PEG	50	50	Once	6 M	3 M
4, 7	rAvPAL–PEG	125	125	Once	6 M	3 M

Study design for assessment of respiratory safety pharmacology of rAvPAL PEG in rats.

[a] rAvPAL-PEG lot #0703070165, vehicle lot #070307FB.

[b] Groups 1–4 were dosed at a volume of 5.95 mL/kg.

Results:

There was no mortality. rAvPAL-PEG had no effects on clinical signs or body weights.

rAvPAL-PEG at 50 or 125 mg/kg produced a mild and dose-dependent decrease in respiratory rate (↓≤ 12%), which elicited a decrease in minute volume (↓≤ 11%) with no change in tidal volume compared with control animals over 6 - 72 hours post-dose. Animals treated with 125 mg/kg rAvPAL-PEG also had a transient but significant decrease (↓28%) in respiratory rate 72 hours post-dose that was not associated with

changes in tidal volume. The changes in respiratory rate and minute volume were considered to be treatment-related. The data for respiratory rate and minute volume is summarized in the tables below (taken from the study report).

Table 13: Mean Respiration Rate Data in SD Male Rats Treated with 10, 50 and 125 mg/kg rAvPAL-PEG

Breaths/minute

Group		Predose	6 hr postdose	24 hr postdose	48 hr postdose	72 hr postdose
			Males			
1	Mean	92	68	88	98	99
	SD	7.0	9.2	15.5	13.8	11.1
	N	6	6	6	6	6
2	Mean	88	81	82	88	87
	SD	15.4	9.0	10.9	7.9	18.5
	N	6	6	6	6	6
3	Mean	80	67	69	88	75
	SD	10.4	7.5	9.3	18.1	13.8
	N	6	6	6	6	6
4	Mean	89	79	79	79	70
	SD	13.6	8.2	11.8	12.2	7.9
	N	6	6	6	6	6

Table 14: Mean Minute Volume Data in SD Male Rats Treated with 10, 50 and 125 mg/kg rAvPAL-PEG

mL/minute

Group		Predose	6 hr postdose	24 hr postdose	48 hr postdose	72 hr postdose
			Males			
1	Mean	203	175	192	211	201
	SD	32.4	35.7	29.6	21.1	34.0
	N	6	6	6	6	6
2	Mean	193	194	197	203	201
	SD	28.1	33.8	48.6	30.6	30.1
	N	6	6	6	6	6
3	Mean	184	162	162	180	166
	SD	17.9	14.1	20.5	21.4	22.2
	N	6	6	6	6	6
4	Mean	213	190	178	181	166
	SD	35.8	31.3	17.5	19.6	16.1
	N	6	6	6	6	6

There was a linear dose-dependent increase in plasma concentrations of rAvPAL-PEG. Maximal concentration (12.34 ± 1.30 µg/mL) was reached at 48 hr following 10 mg/kg rAvPAL-PEG. The maximal concentration for the 50 and 125 mg/kg rAvPAL-PEG was not confirmed as plasma levels continued to increase up to 72 hr post-dose (68.83 + 16.92 µg/mL and 183.61 ± 35.23 µg/mL for 50 and 125 mg/kg, respectively). The T_{max} after a single SC administration in rats was variable and had a broad range (18-96 hours).

In conclusion, a single subcutaneous dose of 10 mg/kg rAvPAL-PEG administered to male SD rats had no effects on respiratory function. However, rAvPAL-PEG at 50 and 125 mg/kg decreased respiratory rate and minute volume. The no observed effect level (NOEL) on the respiratory system was 10 mg/kg.

Effects of rAvPAL-PEG on Cardiovascular System Conscious Telemetry-Instrumented Male Monkeys (Study #: 0165-07-006)

Methods:

Sixteen male cynomolgus monkeys (2.6 to 4.8 years old with body weights from 3.4 to 5.2 kg) were assigned to four groups. Each group received a single dose of the vehicle control article or 1, 3, or 10 mg/kg rAvPAL-PEG via subcutaneous (SC) injection at a dose volume of 0.5 mL/kg. The vehicle was 10 mM Tris and 140 mM NaCl (pH 7.5). The study design is summarized in the table below (taken from study report).

Table 15: Study Design for Cardiovascular Effects in Monkeys

Group	No. of Animals	Dosage (mg/kg)	Concentration (mg/mL)	Dose Volume (mL/kg)
1 (Vehicle control)[a]	4	0	0	0.5
2	4	1	2	0.5
3	4	3	6	0.5
4	4	10	20	0.5

Study design for the assessment of cardiovascular effects in telemetry–instrumented monkeys administered rAvPAL-PEG.

[b] Vehicle control animals received Tris NaCl (0.5 mL/kg) on Day 1 of the dosing phase.

Animals were observed twice daily for mortality, abnormalities, or clinical signs. Qualitative food consumption was assessed once daily. Body weight was recorded three times during the pre-dose phase (including once on the day prior to dosing) and on day 15 following telemetry collection.

Blood samples from all monkeys were collected once on the day prior to dosing and approximately 24 hours post-dose for analysis of plasma rAvPAL-PEG and

phenylalanine. Serum from all monkeys was collected once on the day prior to dosing and day 15 following the final telemetry collection for analysis of anti-rAvPAL-PEG antibody.

At least 2 weeks prior to study initiation, an electrocardiogram transmitter and a dual pressure transmitter (Model No. TL11 M3-D70-PCTP) were implanted into the abdomen and sutured to the abdominal wall. One pressure catheter was placed in an artery and advanced into the abdominal aorta to assess aortic pressure. A second pressure catheter was inserted into the left ventricular chamber of the heart to monitor left ventricular pressures. Data Sciences International (DSI) Dataquest® OpenART® telemetry equipment was used to generate and acquire the cardiovascular data input. This system passed the data to a PONEMAH [P3P (Ponemah Physiology Platform-Plus)] analysis system.

Beginning on day 1 of the dosing phase, ECG, body temperature and hemodynamic measurements were recorded for at least 90 minutes prior to the initiation of dosing and continuously for at least 24 hours after dosing. During the initial 48 hr post-dose, the telemetry recorded for a period of approximately 15 minutes every hour. Thereafter, the telemetry recorded for a period of approximately 15 minutes every 6 hours until day 15 of the dosing phase.

ECG data was collected for evaluation at pre-dose and at 3, 6, 9, and 12 hours post-dose and then at approximately 12-hour intervals thereafter through day 15. The following electrocardiographic parameters were analyzed: heart rate, normal sinus rhythm variations, abnormal sinus rhythms, conductance, repolarization, bradycardia, tachycardia, PR, QRS, QT, QT_{cB} (Bazett's formula) and RR intervals.

Blood pressure measurements included positive inotropic state (dP/dT max), systolic, diastolic, and mean arterial pressures, and arterial pulse pressure (systolic-diastolic). For each dose day, a 5-minute average data was taken for blood pressure assessments twice pre-dose and at approximately 3, 6, 9, and 12 hours post-dose and then at approximately 12-hour intervals thereafter through Day 15.

Intra-abdominal temperature was monitored by telemetry. For each dosing day, a 5-minute average data was taken for body temperature assessments twice pre-dose and at approximately 3, 6, 9, and 12 hours post-dose, and then at approximately 12-hour intervals thereafter through Day 15.

Results:

No mortality, clinical signs of toxicity, or significant changes in body weight were observed.

rAvPAL-PEG had no significant effects on abdominal temperature or hemodynamic parameters (systolic, diastolic, and mean arterial pressures), inotropic state (maximal rate of left ventricular pressure rise [dP/dT max]), or heart rate.

As shown in the Sponsor's tables below, the PR interval was significantly decreased in animals given 1, 3, or 10 mg/kg rAvPAL-PEG compared with that of animals given vehicle across all time points. However, the PR interval did not appear shortened from baseline in these animals, therefore this change was not considered as treatment-related. No drug-related effects on cardiac rhythm, RR, QRS, QT, or QT_c intervals were observed on the 15 day (324 hr). The ECG parameters at selected time points are summarized in the tables below (taken from study report).

Table 16: Mean Electrocardiographic Data in Monkeys Treated with a Single SC Dose of rAvPAL-PEG

		Predose					3 Hour Postdose				
Dose Level (mg/kg)		RR Int (msec)	QRS (msec)	PR Int (msec)	QT Int (msec)	QTc Int (msec)	RR Int (msec)	QRS (msec)	PR Int (msec)	QT Int (msec)	QTc Int (msec)
0	Mean	553	36	77	221	299	562	32	78	218	294
	SD	103.9	1.7	6.2	23.2	21.8	172.9	4.3	4.4	34.7	25.2
	N	4	4	4	4	4	4	4	4	4	4
1	Mean	527	34	74	231	320	498	32	73	218	308
	SD	30.7	2.6	15.9	12.0	16.3	49.3	4.6	18.1	20.0	19.9
	N	4	4	4	4	4	4	4	4	4	4
3	Mean	561	35	75	237	318	575	36	72	230	306
	SD	74.8	1.0	14.0	8.5	18.2	102.2	0.5	12.0	8.7	20.6
	N	4	4	4	4	4	4	4	4	4	4
10	Mean	602	37	68	237	307	583	35	69	227	298
	SD	62.8	5.2	5.0	21.0	17.6	48.1	1.8	7.2	18.2	11.8
	N	4	4	4	4	4	4	4	4	4	4

		12 Hour Postdose					24 Hour Postdose				
Dose Level (mg/kg)		RR Int (msec)	QRS (msec)	PR Int (msec)	QT Int (msec)	QTc Int (msec)	RR Int (msec)	QRS (msec)	PR Int (msec)	QT Int (msec)	QTc Int (msec)
0	Mean	647	36	84	256	318	633	36	78	229	289
	SD	134.9	1.3	7.9	49.7	36.2	87.0	2.7	5.3	14.9	25.4
	N	4	4	4	4	4	4	4	4	4	4
1	Mean	679	38	72	284	345	584	35	76	255	335
	SD	112.0	7.1	14.2	47.2	35.2	64.5	5.1	18.0	14.0	38.8
	N	4	4	4	4	4	4	4	4	4	4
3	Mean	580	34	76	255	336	530	34	72	225	311
	SD	46.4	0.5	14.1	11.0	27.2	107.5	2.9	8.7	18.4	16.1
	N	4	4	4	4	4	4	4	4	4	4
10	Mean	621	37	68	249	317	663	38	69	247	303
	SD	78.1	6.0	2.9	21.4	11.9	108.5	4.3	9.5	28.8	12.1
	N	4	4	4	4	4	4	4	4	4	4

		36 Hour Postdose					60 Hour Postdose				
Dose Level (mg/kg)		RR Int (msec)	QRS (msec)	PR Int (msec)	QT Int (msec)	QTc Int (msec)	RR Int (msec)	QRS (msec)	PR Int (msec)	QT Int (msec)	QTc Int (msec)
0	Mean	542	33	86	234	317	660	36	83	262	321
	SD	39.1	3.0	4.3	35.6	41.7	115.6	5.1	9.9	48.8	36.0
	N	4	4	4	4	4	4	4	4	4	4
1	Mean	693	36	76	288	345	644	35	78	267	334
	SD	133.7	4.3	16.1	51.0	28.6	115.7	5.6	14.6	29.9	19.1
	N	4	4	4	4	4	4	4	4	4	4
3	Mean	606	36	75	252	325	598	37	75	254	329
	SD	115.5	2.4	12.2	22.9	14.1	102.0	3.9	9.8	18.7	12.8
	N	4	4	4	4	4	4	4	4	4	4
10	Mean	639	38	69	242	302	679	38	68	257	313
	SD	117.1	6.7	4.1	34.7	18.9	106.6	4.9	3.4	25.0	19.1
	N	4	4	4	4	4	4	4	4	4	4

Dose Level (mg/kg)		84 Hour Postdose RR Int (msec)	QRS (msec)	PR Int (msec)	QT Int (msec)	QTc Int (msec)	108 Hour Postdose RR Int (msec)	QRS (msec)	PR Int (msec)	QT Int (msec)	QTc Int (msec)
0	Mean	709	37	83	269	318	689	36	86	256	311
	SD	160.4	2.2	6.5	57.6	34.8	158.7	4.7	7.2	57.5	26.2
	N	4	4	4	4	4	4	3	3	3	3
1	Mean	724	36	74	280	329	754	39	76	305	351
	SD	130.3	2.6	14.8	35.7	15.6	172.8	5.5	16.9	51.3	24.9
	N	4	4	4	4	4	4	4	4	4	4
3	Mean	685	35	82	276	334	771	36	78	293	334
	SD	100.2	4.5	14.6	26.4	20.4	92.3	4.5	15.5	12.7	14.5
	N	4	4	3	4	4	4	4	3	4	4
10	Mean	758	38	70	280	322	798	39	67	289	324
	SD	90.8	6.8	3.7	20.5	7.8	111.4	7.4	4.1	25.0	11.3
	N	4	4	4	4	4	4	4	4	4	4

Dose Level (mg/kg)		228 Hour Postdose RR Int (msec)	QRS (msec)	PR Int (msec)	QT Int (msec)	QTc Int (msec)	252 Hour Postdose RR Int (msec)	QRS (msec)	PR Int (msec)	QT Int (msec)	QTc Int (msec)
0	Mean	709	35	83	245	291	693	37	85	265	317
	SD	38.3	3.2	4.3	20.1	27.6	153.7	3.6	6.6	53.4	32.2
	N	4	4	4	4	4	4	4	4	4	4
1	Mean	764	36	76	287	328	681	38	75	268	324
	SD	104.9	3.9	13.3	41.1	28.5	91.6	5.1	14.4	35.1	22.4
	N	4	4	4	4	4	4	4	4	4	4
3	Mean	673	37	78	268	328	661	37	74	255	316
	SD	140.0	2.2	13.1	21.0	12.5	148.4	2.1	11.8	19.5	13.0
	N	4	4	3	4	4	4	4	4	4	4
10	Mean	823	39	69	284	314	776	41	69	271	308
	SD	157.1	5.6	4.3	29.6	7.1	150.4	8.4	3.8	35.8	17.2
	N	4	4	4	4	4	4	4	4	4	4

Dose Level (mg/kg)		324 Hour Postdose RR Int (msec)	QRS (msec)	PR Int (msec)	QT Int (msec)	QTc Int (msec)
0	Mean	848	39	86	294	321
	SD	209.2	4.2	6.1	55.6	37.3
	N	4	4	4	4	4
1	Mean	843	37	80	306	334
	SD	99.2	4.1	14.3	19.8	10.2
	N	4	4	4	4	4
3	Mean	718	38	77	278	329
	SD	116.8	3.2	11.5	24.7	18.4
	N	4	4	4	4	4
10	Mean	866	43	70	298	320
	SD	67.3	6.9	5.4	20.1	13.2
	N	4	4	4	4	4

The increases in plasma concentrations of rAvPAL-PEG were dose proportional. At 24 hr post-dose, rAvPAL-PEG levels were 7949 ± 1490, 11320 ± 3117, and 24200 ± 9400 ng/mL in animals treated with 1, 3, and 10 mg/kg, respectively.

At 24 hr post-dose, rAvPAL-PEG at all doses reduced plasma Phe concentrations to nearly undetectable levels in all animals, except for a single high-dose animal (5.6 µmol/l).

Anti-rAvPAL-PEG IgG was detected in all groups, and the titers appeared to increase in a dose-dependent manner. Anti-rAvPAL IgG with low titers was observed on day 15 in 2/4 (titers: 50-150), 3/4 (titers: 50-450), and 4/4 (titers: 450-1350) monkeys administered 1, 3, and 10 mg/kg, respectively. One and three animals in the 1 and 10 mg/kg groups, respectively, had a positive anti-rAvPAL IgM response at day 15.

In conclusion, male cynomolgus monkeys were administered a single dose of 1, 3, or 10 mg/kg rAvPAL-PEG via SC injection. rAvPAL-PEG had no effects on cardiovascular functions (electrocardiographic or hemodynamic parameters), body temperature, or body weight. All doses of rAvPAL-PEG reduced plasma phenylalanine concentrations within 24 hours post-dose. Anti-drug antibodies (IgG) were present in all animals treated with rAvPAL-PEG, and the titers increased in a dose-dependent manner. A positive anti-rAvPAL IgM response at day 15 was observed in one and three animals in the 1 and 10 mg/kg groups, respectively.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Absorption

60-Day Dose Escalation Pharmacokinetic Study of PEGylated R91K RtPAL (Phenylalanine Ammonia Lyase) and PEGylated AvPAL in Rats (Study #: 0164-06-042)

Methods:

Twenty-four male SD rats (8 weeks old) were divided into 4 groups and administered vehicle (tris-buffered saline, pH 7.5), R91K RtPAL 1:3 PEG (doses of 3.75, 7.5, or 15 mg/kg), AvPAL 1:3 PEG (doses of 3.75, 7.5, or 15 mg/kg), or polyethylene glycol (PEG) (doses of 9.375, 18.75, or 37.5 mg/kg) every 4 days beginning on day 1, for a total of 15 doses via subcutaneous administration.

Clinical observations, body weights and clinical chemistry were evaluated. Postmortem gross examination and organ weights (brain, heart, kidney, liver, lung and spleen) were recorded and organs/tissues (brain, heart, injection sites, kidney, liver, lung and spleen) were collected and examined microscopically. In addition, the left kidneys (glomeruli and tubules) from vehicle control, AvPAL and PEG groups were collected and examined by electron microscopy. Blood samples for pharmacokinetic analysis were collected pre-dose and at 48 and 72 hours post-dose after each dose administration.

Results:

No unscheduled deaths occurred during this study. There were no treatment-related changes in clinical observations, body weights, clinical chemistry, gross exanimations, and organ weights.

The only test article-related histopathological change was perivascular lymphoplasmacytic aggregates (clusters of lymphocytes and plasma cells) around the injection site (1 of 6 in the PEG control group, 6 of 6 in the AvPAL 1:3 PEG group, 4 of 6 in the R91K RtPAL 1:3 PEG group). Thus, PEGylated PAL enhanced the accumulation of perivascular lymphoplasmacytic aggregates at the injection sites.

Fibrosis/scar formation and cellular infiltration in the injection site subcutis, and vacuolation of tubular epithelium in the kidneys were observed in all three treatment groups at similar incidences. Therefore, these effects were likely due to the contribution of PEG.

The electron microscopic examination of kidney showed that increased intraepithelial hyaline droplets in proximal convoluted tubular epithelium was observed in animals from AvPAL and PEG treatment groups, compared to the vehicle control group.

Following the first dose, there was small variation in plasma concentrations of PAL for both R91K RtPAL and AvPAL groups. However, following the 2nd dose and thereafter, the plasma concentrations of PAL varied greatly; a number of animals consistently had plasma concentrations that were below the level of quantitation while others showed increasing concentrations with each dose. Overall, there was a dose-dependent increase in plasma concentrations of R91K RtPAL PEG and AvPAL PEG. Repeated administrations of 15 mg/kg of both R91K RtPAL PEG and AvPAL PEG, but not the doses of 3.75 and 7.5 mg/kg, resulted in increased plasma concentrations.

In general, anti-AvPAL IgG antibody was measurable during the third week of treatment and then increased during the study period in some animals. Animals with strong IgG responses were most likely to have low plasma test article concentrations and animals with no IgG responses showed the highest plasma test article concentrations.

Animals treated with R91K PAL 1:3 PEG (Ig G titers: ranging from below the limit of quantitation to >109350) produced higher titers of antibody compared to animals treated with AvPAL 1:3 PEG (ranging from below the limit of quantitation to 36450).

Single-Dose Pharmacokinetics/Pharmacodynamics of rAvPAL-PEG in Homozygote (-/-) Male BTBRPahenu2 Mice (Study # 0165-07-028)

Methods:

Homozygote (-/-) male ENU2 mice (n = 30) were administered a single dose of 20 mg/ kg rAvPAL-PEG (SC), and blood samples were collected for PK analysis and Phe levels through 240 hr post-dose. Plasma rAvPAL-PEG concentrations were analyzed using a rAvPAL-PEG-specific sandwich enzyme-linked immunosorbent assay (ELISA). The lower limit of quantitation (LLOQ) was 60 ng/mL. The test-article was Phase 1 (b) (4) (b) (4) material.

Results:

The PK data is summarized in the table below (taken from the study report).

Table 17: Plasma Pharmacokinetic Parameters for rAvPAL-PEG Following a Single 20-mg/kg Subcutaneous Injection to Homozygote Male ENU2 Mice

AUC_{0-t} (µg•hr/mL)	$AUC_{0-\infty}$ (µg•hr/mL)	C_{max} (µg/mL)	T_{max} (hr)	$t_{1/2}$ (hr)	V_z/F (mL/kg)	CL/F (mL/hr/kg)
9722	10272	208.613	24	26.9	75.5	1.95

Plasma Phe levels decreased starting at 8 hr post-dose and the Phe levels reached the lowest levels at plasma rAvPAL-PEG concentrations ≥ 50 µg/mL. Serum anti-rAvPAL IgG titers remained at or below detectable limits during the study.

Comparative Single-Dose Pharmacokinetics/Pharmacodynamics for Phase 2 (b) (4) rAvPAL-PEG in Homozygote (-/-) Male and Female BTBRPahenu2 Mice (Study #: 0165-09-069 (Males)*; 0165-09-070 (Females))

Methods:

The two studies were conducted using Phase 2, (b) (4) material of rAvPAL-PEG (pegvaliase). The results from these two studies were compared to the results from study # 0165-07-028 (see above), in which Phase 1, (b) (4) material of pegvaliase was used. Thirty-two male and 28 female ENU2 mice were administered a single dose of 20 mg/kg rAvPAL-PEG (SC). Blood samples were collected pre-dose through 240 hours post-dose to analyze plasma levels of rAvPAL-PEG and Phe. Additional analyses included plasma levels of trans-cinnamic acid (tCA), tyrosine and tryptophan, serum anti-PEG IgG and anti-PEG IgM. Plasma rAvPAL-PEG concentrations were analyzed using a rAvPAL-PEG-specific sandwich enzyme-linked immunosorbent assay (ELISA).

Results:

Pre-dose plasma Phe levels were ~ 1.4-fold higher in females (2.15 ± 0.27 mM) compared to males (1.52 ± 0.15 mM). Females also appeared to have higher rAvPAL-PEG plasma levels following a single 20-mg/kg SC injection. There was an inverse relationship between plasma levels of rAvPAL-PEG and Phe, with a decrease in Phe observed as rAvPAL-PEG levels increased. Phe concentrations appeared to reach a maximal decrease at rAvPAL-PEG plasma concentrations ≥40 µg/mL for both sexes. The Phe levels returned to pre-dose levels by 192 hr post-dose, when rAvPAL-PEG levels were BLQ (60 ng/mL).

The PK parameters for plasma rAvPAL-PEG are summarized in the table below (taken from the study report).

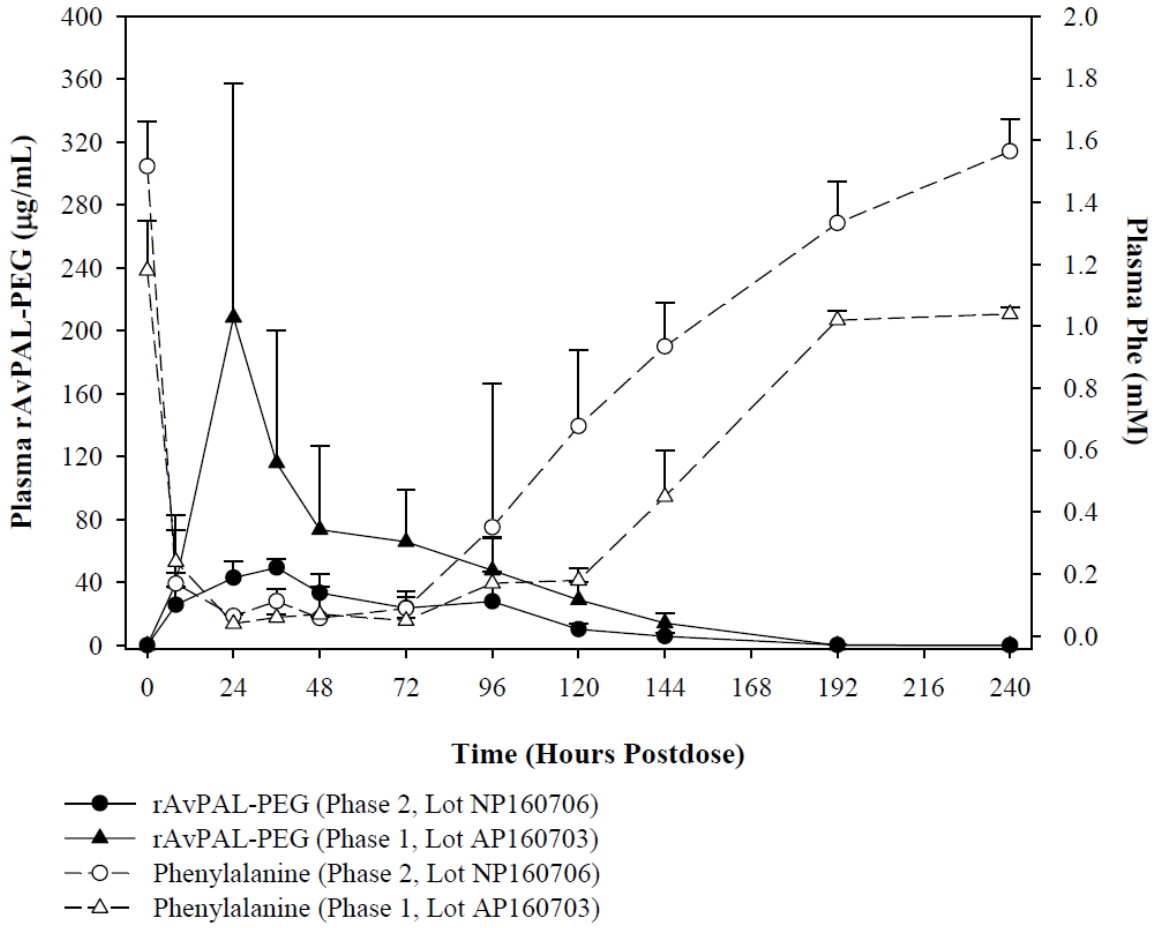
Table 18: Plasma Pharmacokinetic Parameters for rAvPAL-PEG Following a Single 20-mg/kg Subcutaneous Injection to Homozygote ENU2 Mice

Material	Gender	AUC_{0-t} (µg•hr/mL)	$AUC_{0-\infty}$ (µg•hr/mL)	C_{max} (µg/mL)	T_{max} (hr)	$t_{1/2}$ (hr)	V_z/F (mL/kg)	CL/F (mL/hr/kg)
Phase 1	Male	9722	10272	209	24.0	26.9	75.5	1.95
Phase 2	Male	3848	3851	49.4	36.0	11.6	86.7	5.19
Phase 2	Female	11905	11939	171	36.0	16.0	38.6	1.68

Note: Clinical Phase 1 material (Lot No. AP160703, 7.6 monomers PEG/PAL monomer) was previously investigated in males in study # 0165-07-028; a Phase 2 material lot designated for nonclinical use (Lot No. NP160706, 8 monomers PEG/PAL monomer) was investigated in the current study (# 0165-09-069 (males) and 0165-09-070 (females)). Both lots of rAvPAL-PEG were manufactured (b) (4) (b) (4)

Comparison of the PK data from study # 0165-07-028 (clinical Phase 1 material) to PK data for Phase 2 material in the current study demonstrates that exposure (AUC, C_{max}) to Phase 1 material was approximately 3- to 4-fold higher than the Phase 2 material in males. Furthermore, exposure to Phase 2 material was approximately 3- to 3.5-fold higher in females compared to males. Despite the apparent material- and sex-related differences in PK, the PD response profiles for plasma Phe concentration were similar across studies as shown in the table below (taken from the study report).

Figure 10: Mean (+SD) rAvPAL-PEG and Phenylalanine Plasma Levels Following a Single 20-mg/kg Subcutaneous Injection of rAvPAL-PEG (Phase 1/2 (b) (4) Drug Product Comparison) to Homozygote Male ENU2 Mice



Plasma levels of tCA (a Phe metabolite) increased with reduced plasma Phe levels and was observed at the first time point tested (8 hours post-dose). Plasma levels of tCA were higher than Phe through 120 hours post-dose. However, sporadic fluctuation in tCA levels was observed during the 120 hr post-dose. There was no apparent change in plasma tyrosine and tryptophan levels.

For both sexes, almost all animals (except one male and three females) had no detectable antibodies.

Repeat-Dose Pharmacodynamics and Pharmacokinetics of rAvPAL-PEG Administered Subcutaneously Once-Daily for 36 Days in Homozygote (-/-) Male BTBRPahenu2 Mice (Study # 0165-08-006)

Methods:

Eighteen homozygote male ENU2 mice were administered once-daily SC injections of rAvPAL-PEG at 2 mg/kg/day from days 1-14 and at 4 mg/kg/day from days 15-36 followed by a 14-day recovery phase. Blood samples were collected to determine concentrations of rAvPAL-PEG and Phe, and anti-rAvPAL IgG titers. Brains were collected at termination, perfused with saline, and stored for possible future analysis.

Results:

Administration of 2 mg/kg/day rAvPAL-PEG produced a time-dependent increase in plasma rAvPAL-PEG levels with a temporal correlation to decreasing plasma Phe from days 1-8 with no increase in serum anti-rAvPAL IgG titer (≤ 50 TU). However, continuous daily dosing of 2 mg/kg from days 9-14 failed to decrease plasma Phe levels, and plasma Phe concentrations increased to near pretreatment levels. Increasing the dose to 4 mg/kg/day rAvPAL-PEG from days 15-36 resulted in generally decreasing plasma Phe levels during days 19-37. Plasma Phe levels increased to pretreatment baseline levels after termination of treatment. The loss of pharmacological effect on days 9-16 was possibly due to increased clearance or neutralization of rAvPAL-PEG.

Anti-rAvPAL IgG titers (≤ 450 TU) remained near minimum detectable levels throughout the dosing and non-dosing phases with one exception during the treatment period.

As shown in the table below (taken from the study report), after dosing with 2 mg/kg on Day 1 and 4 mg/kg on Day 36, plasma concentrations of rAvPAL-PEG steadily increased and reached a C_{max} at 24 hours post-dose.

Table 19: Plasma Pharmacokinetic Parameters for rAvPAL-PEG Following Once-Daily SC Injections to Male ENU2 Mice for 36 Days

Dose Day	Dose (mg/kg)	T_{lag} (hr)	T_{max} (hr)	C_{max} (ng/mL)	T_{last} (hr)	C_{last} (ng/mL)	AUC_{0-24} (ng•hr/mL)	AI (-fold)
1	2	0	24	16133	24	16133	110366	--
36	4	0	24	66527	24	66527	702860	3.2

Note: rAvPAL-PEG was given at 2 mg/kg/day on Days 1-14 and at 4 mg/kg/day on Days 15-36.
AI: Accumulation index, corrected for dose difference from Day 1 to Day 36 (Section 6.10).

rHuPH20/rAvPAL-PEG Testing in Rats Summary Report (Study #: 165-11-018)

The objective of this study was to evaluate whether rHuPH20, recombinant human hyaluronidase which can digest hyaluronic acid, can increase the bioavailability of rAvPAL-PEG. SD male rats were divided into 5 groups (n = 10). Groups 1 and 2 were treated with a single dose 5 mg/kg/day rAvPAL-PEG via intradermal (ID) injection; groups 3 and 4 were treated with a single dose of 5 mg/kg/day rAvPAL-PEG and 8.5 µg/kg/day rHuPH20 via ID administration; group 5 was treated with a single dose of rAvPAL-PEG at 1 mg/kg via IV administration. Based on AUC_{last}, rHuPH20 enhanced rAvPAL-PEG's bioavailability by about 10%. rHuPH20 increased the absorption rate for rAvPAL-PEG (T_{max} was 32-35 hr, compared to 61 hr without rHuPH20).

5.2 Toxicokinetics

(See General Toxicology section)

6 General Toxicology

6.1 Single-Dose Toxicity

Single-Dose Subcutaneous or Intravenous Injection Toxicity and Toxicokinetic Study with rAvPAL-PEG in Rats with a 2-Week Recovery Period (Study #: 0165-07-003)

SD rats were given rAvPAL-PEG either as an intravenous administration at a single dose of 1, 5, or 25 mg/kg or as a subcutaneous administration at a single dose of 10, 25, or 250 mg/kg. Brain, heart, kidneys, injection sites, lesions (if present), liver and lungs were subjected to histopathologic examination. rAvPAL-PEG-related findings for animals given 250 mg/kg subcutaneously included red or scaly skin, decreased body weight and body weight gain, decreases in red cell mass, absolute reticulocyte count, absolute neutrophil count, and total protein, vacuolation of Kupffer cell in liver, and/or macrophage infiltrates and subacute inflammation at the subcutaneous injection site. Animals receiving 25 mg/kg IV rAvPAL-PEG had the same changes in hematology and clinical chemistry parameters as that of the animals receiving 250 mg/kg SC rAvPAL-PEG. Decreased urinary volume and higher urine specific gravity were observed in males and females in the 25 mg/kg IV and males in the 250 mg/kg SC groups. All animals in these two groups had decreased urinary pH.

Systemic exposure (AUC_{0-96} and C_{max} values) were approximately dose proportional for both the IV and SC routes of administration at lower doses (1, 5 mg/kg IV doses or 10, 25 mg/kg SC doses) and were less than dose proportional at the highest dose. After SC administration, rAvPAL-PEG was slowly absorbed with T_{max} values between 12 and 120 hours. The bioavailability of rAvPAL-PEG after SC administration ranged from 7.48 to 21.9%, indicating a relatively low absorption by this route of administration. There was no clear sex difference in systemic exposure after IV or SC administration. Dose levels up to 5 mg/kg IV and 25 mg/kg SC were tolerated.

Single-Dose Subcutaneous Injection Toxicity and Toxicokinetic Study with rAvPAL-PEG in Cynomolgus Monkeys with a 3-Week Recovery (Study #: 0165-07-007)

This was a GLP study. Cynomolgus monkeys (3/sex/dose group and 3 to 4.5 years old) received a single SC injection of rAvPAL-PEG at a dose of 0 (control vehicle), 4, 12 or 60 mg/kg with a dose volume of 2.86 mL/kg. All monkeys were observed for three weeks. The control article was 10 mM trishydroxymethylaminomethane (Tris), 140 mM sodium chloride (pH 7.5).

A full necropsy was performed on all animals. A subset of organs (adrenals, brain, epididymides, heart, kidneys, liver, lungs, ovaries, spleen, testes and thymus) was weighed. The brain, heart, kidneys, injection sites, lesions, stomach tissues, liver and lungs were subjected to histopathological examinations.

Table 20: Summary of Key Findings in a Single Dose Toxicity Study in Monkeys via SC injection

Groups	Dose (mg/kg)	Key Findings
1	**4**	↓phenylalanine (Phe) within 12 hr through 264-360 hr (11-15 days) post-dose
2	**12**	One male had no visible food consumption and had watery mucoid feces on day 6, and was treated with SC fluid therapy, and appeared normal by day 8. ↓Phe within 12 hr through 18 days post dosing (the final test interval). Mild-moderate decreases in following hematology parameters: ↓Absolute reticulocyte count (240 hr), ↓RBC count, Hgb, Hct (48 and 240 hr), and MCV (240 hr). All the changes were comparable to control values on day 23.
3	**60**	All animals were euthanized on day 6 or 7 due to moribund condition. The cause of moribundity is likely related to the gastrointestinal tract mucosa alteration (see below). Clinical signs included depression, dehydration, anorexia, hypothermia/cold to touch, diarrhea, hunched posture, lump at injection site, hypoactivity, vomitus containing food, rough hair coat, squinting eyes, thin appearance, and pale skin. Weight loss (↓7%-17% in males; ↓13%-15% in females). ↓Phe by 12 hr through termination. Mild-moderate decreases in following hematology parameters: ↓absolute reticulocyte count, ↓absolute lymphocyte and eosinophil count, ↓Alkaline phosphatase, ↓ total protein (due to decreased albumin and globulin)

		↑BUN ↓Serum pre-albumin concentration • Macroscopic GI lesions or discolorations and blood in tract (3M, 3F) • Stomach pyloric mucosal ulcerations up to 1 cm^2 (2M, 3F) • Discoloration on mucosal surface of distal colon or ileocecal junction (3M, 0F) • Raised, dark red lesions up to 0.5 cm^2 on serosal surface of proximal colon (1M, 0F) • Histopathological changes in gastrointestinal tract (stomach, ileum, and cecum) included mucosal atrophy, congestion and hemorrhage, degeneration and necrosis of glandular epithelium, erosion, ulceration, and subacute inflammation.

Table 21: Mean Toxicokinetic Parameters for rAvPAL-PEG in Monkey Plasma after a Single SC Administration (Continued)

Dose Group	Dose Level (mg/kg)	Sex		C_{max} (ng/mL)	DN C_{max} [(ng/mL) / (mg/kg)]	T_{max} (hr)	AUC_{0-t} (ng•hr/mL)	AUC_{0-24} (ng•hr/mL)	DN AUC_{0-24} [(ng•hr/mL) / (mg/kg)]	$AUC_{0-\infty}$ (ng•hr/mL)	$t_{½}$ (hr)	Cl/F (mL/hr/kg)	V_z/F (ml/kg)
4	60	M	Mean	217280	3621	52.0	6885560	782460	13041	NA	NA	NA	NA
			SD	41942	699	38.6	8147343	373125	6219	NA	NA	NA	NA
			N	3	3	3	3	3	3	0	0	0	0
		F	Mean	219493	3658	80.0	11753160	562520	9375	NA	NA	NA	NA
			SD	45288	755	34.6	5655311	223247	3721	NA	NA	NA	NA
			N	3	3	3	3	3	3	0	0	0	0
		Combined	Mean	218387	3640	66.0	9319360	672490	11208	NA	NA	NA	NA
			SD	39058	651	36.2	6815626	300228	5004	NA	NA	NA	NA
			N	6	6	6	6	6	6	0	0	0	0

Note: The combined is the mean of the males and females.

In the single dose SC toxicity study in cynomolgus monkeys, rAvPAL-PEG at all doses deceased plasma phenylalanine, an effect that was consistent with its pharmacologic activity. rAvPAL-PEG at a dose of 60 mg/kg caused moribund condition due to gastrointestinal tract ulcerations, hemorrhage and degeneration and necrosis of glandular epithelium. At doses of 12 and 60 mg/kg, rAvPAL-PEG decreased reticulocyte count, RBC, hemoglobin, lymphocyte and eosinophil count, and total protein (due to decreased albumin and globulin). In general, exposure to rAvPAL-PEG (C_{max} and AUC_{0-24}) in both sexes increased with dose in a less than dose proportional manner, except for C_{max} in males at 12 mg/kg. There was no sex-related difference in systemic exposure. The $t_{1/2}$ of rAvPAL-PEG administered as a single SC injection ranged from 64.5 hours (at 4 mg/kg) to 50.6-92 hours (at 12 mg/kg). After SC administration, rAvPAL-PEG was slowly absorbed. The target organ of toxicity was the gastrointestinal tract (12 and 60 mg/kg). rAvPAL-PEG given at 4 mg/kg was well tolerated.

6.2 Repeat-Dose Toxicity

Study title: 4-Week Subcutaneous Injection Toxicity and Toxicokinetic Study with rAvPAL-PEG in Rats with a 2-Week Recovery Phase

Study no.:	0165-07-009
Study report location:	N/A
Conducting laboratory and location:	(b) (4)
Date of study initiation:	April 30, 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	NP160701; purity: 99.5%; Specific activity: 1.4 U/mg

Key Study Findings

SD rats were treated with rAvPAL-PEG at doses of 0, 1, 8, and 25 mg/kg twice weekly via subcutaneous (SC) injection for 28 days. rAvPAL-PEG had no adverse effects on body weight, food consumption, hematology, clinical chemistry, or urinalysis. rAvPAL-PEG at doses of 8 and/or 25 mg/kg produced vacuolation of Kupffer cells in liver and reticuloendothelial cells in spleen. However, these findings were not adverse. Overall, there was a dose proportional increase in systemic exposure to rAvPAL-PEG. No accumulation of rAvPAL-PEG was observed following repeated doses. In general, females had higher C_{max} and AUC_{0-t} values than males. IgG anti-drug antibody was detected in some animals, but the incidence and titer values were not dose-dependent. The NOAEL was 25 mg/kg/dose.

Methods

Doses:	0 (vehicle), 1, 8, and 25 mg/kg
Frequency of dosing:	Twice weekly
Route of administration:	Subcutaneous injection (SC) in the scapular region (site A), and the lumbar region (site B)
Dose volume:	1.22 ml/kg
Formulation/Vehicle:	rAvPAL-PEG was dissolved in vehicle (10 mM TRIS, 140 mM sodium chloride (NaCl), pH 7.5 ± 0.1) at concentrations of 0.82, 6.56, and 20.5 mg/ml.
Species/Strain:	Crl:CD(SD) rats
Number/Sex/Group:	10-15/sex/group
Age:	46 to 52 days old
Weight:	192 to 267 g for males and 147 to 226 g for females
Satellite groups:	3-6 sex/group for TK and antibody analysis
Unique study design:	Not applicable
Deviation from study protocol:	Minor deviations had no impact on data integrity or interpretation of results.

Table 22: Study Design for a 28-day SC Toxicity Study in Rats

Group	No. of Animals Male	No. of Animals Female	Dose Level (mg/kg)	Dose Volume (mL/kg)	Dose Concentration (mg/mL)
Toxicity Animals					
1 (Control)[c,d]	15	15	0	1.22	0
2 (Low)	10	10	1	1.22	0.82
3 (Mid)	10	10	8	1.22	6.56
4 (High)[a,d]	15	15	25	1.22	20.5
Toxicokinetic Animals[b]					
5 (Control)[c]	3	3	0	1.22	0
6 (Low)	6	6	1	1.22	0.82
7 (Mid)	6	6	8	1.22	6.56
8 (High)[a]	6	6	25	1.22	20.5

a Nominal concentration, provided by sponsor.
b Toxicokinetic animals included solely for the purpose of blood sample collections.
c Groups 1 and 5 received control article only.
d Animals designated for recovery phase sacrifice (five animals/sex in Groups 1 and 4) underwent at least 2 weeks of recovery following dose administration.

Observations and Results

Mortality

Mortality was checked twice daily. No deaths occurred during the treatment and recovery periods.

Clinical Signs

Clinical signs were monitored twice daily. There were no treatment-related clinical signs.

Body Weights

Body weight was recorded weekly. Bodyweight was reduced by up to 5% in males treated with rAvPAL-PEG, but the reduction was not dose-dependent and did not reach statistical significance. rAvPAL-PEG had no adverse effects on body weight or body weight gain. The body weights are summarized in the table below.

Table 23: Body Weights in a 28-Day SC Toxicity Study in Rats

Dose Groups (mg/kg/dose)	Body Weights (g)			
	Day 1		Day 27	
	Males	Females	Males	Females
0	236	189	421	261
1	235	188	409	254
8	234	190	400	263
25	235	189	405	257

Feed Consumption

Food consumption was measured daily. rAvPAL-PEG had no adverse effects on food consumption.

Ophthalmoscopy

Ophthalmoscopy was performed pre-dose for all animals and during the final week of the dosing phase for main study animals. There were no treatment-related changes.

ECG

N/A

Hematology

Blood samples were collected from fasted animals in the main study groups at scheduled sacrifice.

There were no treatment-related changes in hematology parameters except for a 6% increase in APTT in the high-dose groups (22.5 ± 1.26 sec in males vs. 21.2 ± 0.89 sec in controls; 21.4 ± 0.77 sec in females vs. 20.3 ± 1.05 sec in controls; $p<0.05$). No changes were noted at the end of the recovery period.

Clinical Chemistry

Blood samples were collected from fasted animals in main study groups at scheduled sacrifice. There were no treatment-related changes in clinical chemistry.

Urinalysis

Urine was collected overnight before blood collection. No changes were observed in urinalysis including volume, specific gravity, and pH.

Gross Pathology

All main study animals were subjected to complete necropsy. There were no test article-related changes in macroscopic findings.

Organ Weights

The following organs were weighed: adrenal, brain, epididymis, heart, kidney, liver, lung, ovary, spleen, testis, and thymus. There were no test article-related changes in absolute or relative organ weights.

Histopathology

Adequate Battery: Yes

Peer Review: No

Preserved tissues were examined microscopically from all animals. The following tissues from each animal were preserved in 10% neutral buffered formalin, unless otherwise indicated below (a: preserved in modified Davidson's fixative): adrenal, aorta, brain, cecum, cervix, colon, duodenum, epididymis[a], esophagus, eyes[a], femur with bone marrow (articular surface of the distal end), heart, ileum, injection site(s), jejunum, kidney, lesions, liver, lung with large bronchi, lymph node (mandibular and mesenteric), mammary gland (females), optic nerve[a], ovary, pancreas, pituitary gland, prostate, rectum, salivary gland (mandibular), sciatic nerve, seminal vesicle, skeletal muscle (thigh), skin/subcutis, spinal cord (cervical, thoracic, and lumbar), spleen, sternum with bone marrow, stomach, testis[a], thymus, thyroid with parathyroid, tongue, trachea, urinary bladder, uterus, and vagina.

Histological Findings

Treatment-related histopathologic changes are summarized in the table below. rAvPAL-PEG at 25 mg/kg increased the incidence in vacuolation of liver Kupffer cells compared to controls. Mid- and high-dose animals had minimal vacuolation in reticuloendothelial cells in spleen. Minimal vacuolation was still observed at the recovery phase in the liver of two control males and in the spleen of three males and four females in the high-dose groups.

Test article-related microscopic findings at the subcutaneous injection sites were minimal to marked fibrosis and minimal to slight infiltrates of lymphocytes/macrophages. Fibrosis was more frequently observed in animals in the mid- and high-dose groups. The findings at the subcutaneous injection sites were reversed after the recovery phase.

Table 24: Treatment-Related histopathologic changes in a 28-Day SC Rat Toxicity Study

	Males				Females			
Dose (mg/kg)	**0**	**1**	**8**	**25**	**0**	**1**	**8**	**25**
Number	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Liver								
Infiltrate, lymphocyte/macrophage	8	8	8	4	5	4	7	7
Vacuolation in Kupffer cells (minimal)	1	0	0	5	2	0	0	3
Spleen								
Minimal vacuolation in reticuloendothelial cells	0	0	4	6	0	0	0	2

Toxicokinetics

Blood samples from all TK animals were collected on days 1 (Dose 1), 11 (Dose 4), and 22 (Dose 7) at pre-dose and at approximately 3, 6, 9, 12, 24, 36, 48, 60 and 72 hours post-dose. Due to variable plasma concentration profiles and many individual samples below the lower limit of quantitation (250 ng/mL), the data should be interpreted with caution across all days and dose levels.

Overall, systemic exposure to rAvPAL-PEG was generally dose proportional. There was no accumulation of rAvPAL-PEG following repeated doses for 22 days (7 doses). After SC administration, rAvPAL-PEG was slowly absorbed and eliminated. In general, females had higher rAvPAL-PEG C_{max} and AUC_{0-t} values than males. Increases in rAvPAL-PEG C_{max} and AUC_{0-t} for both sexes were roughly dose proportional on day 1. The toxicokinetic parameters for rAvPAL-PEG are summarized in table below (taken from study report).

Table 25: Toxicokinetic Parameters for rAvPAL-PEG in Rat Plasma in a 28-Day SC Toxicity Study

Dose Group	Dose Level (mg/kg/dose)	Sex	C_{max} (ng/mL)	DN C_{max} [(ng/mL) / (mg/kg/dose)]	T_{max} (hr)	AUC_{0-t} (ng•hr/mL)	DN AUC_{0-t} [(ng•hr/mL) / (mg/kg/dose)]
Day 1 (Dose 1)							
6	1	M	1932	1932	36.0	72350	72350
		F	1938	1938	24.0	80425	80425
		Combined	1935	1935	30.0	76388	76388
7	8	M	28213	3527	36.0	1241165	155146
		F	25652	3206	48.0	968380	121048
		Combined	26933	3367	42.0	1104773	138097
8	25	M	61353	2454	60.0	2680523	107221
		F	83893	3356	60.0	3304015	132161
		Combined	72623	2905	60.0	2992269	119691
Day 11 (Dose 4)							
6	1	M	230	230	0	2465	2465
		F	NA	NA	NA	NA	NA
7	8	M	107	13.3	24.0	1365	171
		F	468	58.5	9.00	7818	977
		Combined	288	35.9	16.5	4591	574
8	25	M	5168	207	36.0	168338	6734
		F	36960	1478	12.0	1588160	63526
		Combined	21064	843	24.0	878249	35130
Day 22 (Dose 7)							
6	1	M	NA	NA	NA	NA	NA
		F	NA	NA	NA	NA	NA
7	8	M	5882	735	36.0	169018	21127
		F	4403	550	9.00	147790	18474
		Combined	5143	643	22.5	158404	19800
8	25	M	35773	1431	36.0	1386565	55463
		F	77087	3083	36.0	4123605	164944
		Combined	56430	2257	36.0	2755085	110203

Note: The combined is the mean of the males and females.

Special Evaluation

1. Electronic microscopic (EM) examination

Samples of the kidney cortex and medulla were collected from all animals at scheduled sacrifice and preserved in McDowell Trump fixative for EM. rAvPAL-PEG had no effects on kidney ultrastructure at the end of the treatment and recovery periods.

2. Antibody analysis

Blood samples were collected from all TK animals once during the pre-dose phase and on days 10, 14, 22, and 28 of the dosing phase, once during weeks 1 and 2 of the recovery phase, and at termination.

IgG rAvPAL antibodies were first detected in one female in the 1 mg/kg/dose group on day 10. The antibody titer levels and incidence of anti-rAvPAL IgG titers after rAvPAL-PEG administration were not dose-dependent and were not observed in all animals. The highest titer was observed in female rats administered 1 mg/kg (SC). In general, female rats had relatively higher titers than males. The number of rats that were IgG antibody positive and their titers is summarized in the table below. IgM anti-drug antibody was observed in two females in the 1 mg/kg/dose group on day 22 of the dosing phase and at recovery week 2.

The antibody titers generally correspond with the disappearance of plasma rAvPAL-PEG levels and may explain the decreased exposure levels in rats over time and the lack of expected plasma accumulation after repeated administrations.

Table 26: Numbers of Rats with IgG Antibody Positive and Antibody Titer Levels in a 28-Day SC Rat Toxicity Study

Time-points	**Antibody**	**Dose Groups (mg/kg/dose)**		
On Day 28 (at the end of treatment period)		**1**	**8**	**25**
Males (n = 6)	IgG positive rats	**2**	**1**	**2**
	Titers	< 50 - 450	<50 - 50	< 50 - 150
Females (n = 6)	IgG positive rats	**4**	**1**	**3**
	Titers	< 50 - 12150	<50 - 150	< 50 - 150
On Day 42 (at the end of the recovery period)				
Males (n = 6)	IgG positive rats	**2**	**1**	**5**
	Titers	< 50 - 450	<50 - 50	<50 - 450
Females (n = 6)	IgG positive rats	**5**	**0**	**4**

	Titers	< 50 - 12150	<50	<50 - 1350

Dosing Solution Analysis

Duplicate samples were collected for homogeneity analysis from the top, middle, and bottom of the low- and high-dose solutions for doses 1 and 8. All homogeneity samples were within 5% of the mean concentration for that group. Therefore, the 0.82 and 21 mg/mL concentrations were homogenous.

The stability of the low- and high-dose solutions for day 1 was analyzed. Duplicate samples were collected on the day of dose preparation within approximately 5 minutes of the end of preparation (thawing in the case of the high-dose, and dilution in the case of the low-dose), and at approximately 3 hours after preparation with storage at room temperature. There was no noticeable change in activity in solutions containing either 0.82 or 21 mg/mL rAvPAL-PEG, after the solutions were maintained at room temperature for three hours prior to freezing and storage. The dose solutions were considered to be stable.

Study title: 26-Week Subcutaneous Injection Toxicity and Toxicokinetic Study with a 12-Week Recovery Period and a 17-Week Interim Euthanasia with a 4-Week Recovery Period in Rats

Study no.:	0615-08-019
Study report location:	N/A
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 1, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	see table below

Table 27: Lot Numbers and Purities

Test Article	Lot No.	Storage	Purity	Expiration Date	Reserve (Archive) Sample[a]
rAvPAL-PEG	FIN-0426	Vials were stored in a refrigerator, set to maintain 2 to 8°C[a]	100%	01 Aug 2008	Collected
	AP160802	Bulk test article [intravenous (IV) bags] was stored in a freezer, set to maintain -60 to -80°C (test article received 04 Jun 2008) or in a refrigerator, set to maintain 2 to 8°C (test article received 23 Sep 2008)[a]	100%	13 Mar 2009	Collected
	AP160703	Bulk test article (IV bags) was stored in a freezer, set to maintain -60 to -80°C.	100%	19 Jul 2008	Collected
	NP160801	Bulk test article (IV bags) was stored in a freezer, set to maintain -60 to -80°C[a]	100%	28 Feb 2009	Collected

a See Protocol Deviations.

Key Study Findings

SD rats were treated with rAvPAL-PEG at doses of 0, 1, 8, and 25 mg/kg twice weekly via subcutaneous injection for 17 and 26-weeks. rAvPAL-PEG at 25 mg/kg/dose decreased body weight (approximately 6% for both sexes) and body weight gain (up to 17% for males and 34% for females). The decreased body weight and body weight gain in males were associated with reduced food consumption (up to 6%). Following the 17- and 26-week treatment periods, similar treatment-related histopathological changes were observed in the kidney, spleen, liver, testes, adrenal gland, mesenteric and mandibular lymph nodes, and subcutaneous injection sites in animals in the 8 and/or 25 mg/kg/dose groups. These changes included focal to multifocal areas of vacuolation/hypertrophy of renal tubule cells, increased vacuolation in histiocytic cells in the adrenal cortex, liver, spleen, mesenteric and mandibular lymph node, and testes. Incidence and severity of these changes increased with duration and dose level. At the end of the 12-week recovery phase, all of these changes persisted but with less severity. Test article-related microscopic findings at subcutaneous injection sites included minimal to marked infiltrates, vacuolated histiocytic cells and lymphocytes, fibrosis, minimal degeneration of dermis, skeletal muscle, and/or adipocytes. The local effects were mostly limited to the 25 mg/kg group, and may be considered as adverse. The target organ of toxicity was kidney, based on vacuolation/hypertrophy of renal tubule cells that persisted through the 12-week recovery period in groups treated with 8 or 25 mg/kg. Systemic exposure to rAvPAL-PEG generally increased in a dose proportional manner. No accumulation of rAvPAL-PEG in plasma occurred following repeated doses. In general, females had higher C_{max} and AUC_{0-t} values than males. IgG anti-drug antibody was detected in most animals. The NOAEL was 1 mg/kg twice weekly.

Methods

Doses:	0 (vehicle), 1, 8, 25 mg/kg
Frequency of dosing:	Twice weekly
Route of administration:	Subcutaneous injection (the first 33 or 53 injections were dosed in the scapular region (site A), while the final dose was injected in the lumbar region (site B))
Dose volume:	2.5 ml/kg
Formulation/Vehicle:	rAvPAL-PEG was dissolved in control article/diluent (10 mM tris (hydroxymethyl) aminomethane (TRIS), 135 mM sodium chloride (NaCl), 1 mM trans-cinnamic acid (tCA), and 1 mM ammonia) at concentrations of 0.4, 3.2, and 10 mg/ml.
Species/Strain:	Crl:CD(SD) rats
Number/Sex/Group:	25-35/sex/group
Age:	49 to 55 days old
Weight:	227 to 298 g for males and 162 to 214 g for females
Satellite groups:	9/sex/group for TK study
Unique study design:	N/A
Deviation from study protocol:	Deviations had no impact on data interpretation.

Table 28: Study Design for a 26-Week SC Toxicity Study in Rats

Group	No. of Animals Male	No. of Animals Female	Dose Level (mg/kg/dose)	Dose Concentration (mg/mL)
Toxicity Animals				
1 (Control)[c,d,e]	35	35	0	0
2 (Low)[d,e]	25	25	1	0.4
3 (Mid)[d,e]	25	25	8	3.2
4 (High)[a,d,e]	35	35	25	10
Toxicokinetic Animals[b]				
5 (Control)[c]	9	9	0	0
6 (Low)	9	9	1	0.4
7 (Mid)	9	9	8	3.2
8 (High)[a]	9	9	25	10

a Nominal concentration, provided by sponsor.
b Toxicokinetic animals included solely for the purpose of blood sample collections.
c Groups 1 and 5 received control article only.
d Animals designated for interim euthanasia (10 animals/sex/group) were euthanized after 17 weeks of dose administration. Animals designated for interim recovery euthanasia (five animals/sex/group, control and high-dose groups) underwent 4 weeks of recovery following 17 weeks of dose administration.
e Animals designated for terminal euthanasia (up to 15 animals/sex/group, dependent on survival) were euthanized after at least 26 weeks of dose administration. Animals designated for recovery euthanasia (five animals/sex/group, control and high-dose groups) underwent 12 weeks of recovery following at least 26 weeks of dose administration.

Observations and Results

Mortality

All animals were monitored twice daily. There were a total of 4 unscheduled deaths. None of the deaths were test article-related.

Mortality in Interim Phase (initial 17 weeks of treatment)

One male in the 1 mg/kg/dose group was found dead on day 71, and one male in the TK group given 1 mg/kg/dose was euthanized on day 106. There were no treatment-related clinical signs or microscopic findings in the male found dead. The male euthanized had few feces, red discharge from the left eye, and malocclusion. The deaths were not considered to be related to the test article.

Mortality during weeks 18-26 of treatment

Two males in the 1 mg/kg/dose group were euthanized on days 133 and 164. The male euthanized on day 133 had convulsions lasting more than one minute, clear oral discharge, hyperactivity, and vocalization. The moribund condition was due to

inflammation of the pancreas. The male euthanized on day 164 had few feces, red discharge from the eyes, hypoactivity, cold body, and general debilitation. The death was due to brain neoplasm.

Clinical Signs

All animals were checked twice daily. During the first 17 weeks of the dosing phase, 17/35 females in the 25 mg/kg/dose group had thinning hair coat at the injection site or the dorsal abdomen (back). During the final dosing phase (days 120 through 182), thinning hair coat at the injection site or the dorsal abdomen was observed in 10/20 females in the 25 mg/kg/dose group. Thin hair coat was considered test article-related. No treatment-related clinical signs were observed during the recovery period.

Body Weights

Body weights were recorded weekly. rAvPAL-PEG at 25 mg/kg/dose decreased body weight and body weight gain in males and females. During the initial 17-week treatment period, body weight reduction in males was more evident compared to the rest of the treatment period (days 120 to 182). A significant decrease in male body weight was observed through day 71 to day 99 (↓up to approximately 6%; $p \leq 0.05$). However, females in the 25 mg/kg/dose group had less reduction in body weight compared to the males during the first 17-week treatment period. No significant body weight reduction was observed in females in the 25 mg/kg/dose group during the 26-week treatment period. The maximum reduction in female body weight was 5.7% at the end of the treatment period. The body weight changes in males and females are summarized in tables below.

Table 29: Body Weight Changes in Males in a 26-Week Rat Toxicity Study

Treatment Period	Male Dose Groups (mg/kg/dose)			
	0	**1**	**8**	**25**
Day 1	258	257	258	258
Day 85	531	517	530	500*
Day 120 (week 17)	546	536	546	517
4-Week Recovery period				
Day 4	561	-	-	548
Day 32	562	-	-	558
26-Week Treatment Period				
Day 127	568	569	576	548
Day 183 (week 26)	624	616	621	596
12-Week Recovery Period				
Day 4	619	-	-	621
Day 81	635	-	-	645

*: $p \leq 0.05$

Table 30: Body Weight Changes in Females in a 26-Week Rat Toxicity Study

Treatment Period	Female Dose Groups (mg/kg/dose)			
	0	1	8	25
Day 1	186	187	186	186
Day 85	303	305	302	295
Day 120 (week 17)	307	309	305	296
4-Week Recovery Period				
Day 4	290	-	-	318
Day 32	285	-	-	315
26-Week Treatment Period				
Day 127	320	321	321	311
Day 183 (week 26)	348	347	348	328
12-Week Recovery Period				
Day 4	368	-	-	327
Day 81	386	-	-	350

The body weight gain in males in the 25 mg/kg/dose group was 91.1% of the control males during the 17-week treatment period. However, the mean body weight gain in the high-dose group further decreased and was 82.9% of the controls at the end of the 26-week treatment. In addition, the overall mean body weight gain (days 120 to 183 of the dosing phase) for females given 25 mg/kg/dose was 65.7% of the control females ($p \leq 0.05$). The body weight and body weight gain were comparable at the end of the 4-week or 12-week recovery period (see tables above). Thus, rAvPAL at a dose of 25 mg/kg/dose reduced body weight and body weight gain in both sexes.

Feed Consumption

Food consumption was measured weekly. Males in the 25 mg/kg/dose group had lower food consumption than control males through the initial 17-week treatment period. Significant reduction in food consumption was observed in multiple time points (↓up to 11% on day 78 - 84; $p \leq 0.05$). Through the rest of the treatment period (days 120 to 182), males in the 25 mg/kg/dose group also had lower food consumption (↓up to 6%), but this change did not reach statistical significance. rAvPAL had no effects on food consumption in females. The reduced food consumption in the 25 mg/kg/dose male group is considered as treatment-related. During the 4-week recovery period, the food consumption in animals in the 25 mg/kg/dose group was comparable to the controls. However, in general, males in the 25 mg/kg/dose group had increased food consumption (↑up to 7%, $p \leq 0.05$) during the 12-week recovery period.

Ophthalmoscopy

Ophthalmic examinations were conducted during the pre-dose phase, during weeks 17 and 26 of the dosing phase, and during weeks 5 and 12 of the recovery phase. One of 25 males in the 1 mg/kg/dose group and one of 35 males in the 25 mg/kg/dose group had corneal dystrophy in the left eye at the end of the 17-week treatment period.

Vitreous hemorrhage in the right eye was observed in one of 35 males in the 25 mg/kg/dose group at the end of the 26-week treatment period. No lesions were observed in animals at the end of the 4-week or 12-week recovery period. Due to the low incidence of ophthalmic changes and absence of a dose-dependent effect, the ophthalmic changes are not considered as treatment-related.

Hematology

Blood samples from fasted animals for hematology analysis were collected during the treatment period (days 89, 117, and 187) and recovery period (days 148, 211, 246, and 272). There were no treatment-related changes.

Clinical Chemistry

Blood samples from fasted animals for clinical chemistry were collected during the treatment period (days 22, 50, 89, 117, and 187) and recovery period (days 148, 211, 246, and 272). Treatment-related changes included slight or moderate decreases in triglycerides (↓20-39%) in the 25 mg/kg/dose group, alanine aminotransferase (ALT) in females (↓25-30%) at 8 mg/kg/dose and in males and females (↓18-35%) at 25 mg/kg/dose, and alkaline phosphatase (ALP) in males (↓14%) in the 25 mg/kg/dose group. The reduction in triglycerides were reversed at the end of the 4- and 12-week recovery periods, but not ALT (↓20%) and ALP (↓21-23%).

Urinalysis

Urine samples were collected from fasted animals during the pre-dose phase, treatment period (days 22, 50, 89, and 117) and recovery period (days 148, 187, 211, 2462, 272). Males in the 25 mg/kg/dose group had lower urine pH during the 17- and 26-week treatment periods (6.5 vs. 6.7 in the controls; $p \leq 0.05$). The change was not observed at the end of the 4- or 12-week recovery period. Significantly decreased urine pH was also observed in the females treated with 8 or 25 mg/kg/dose at the end of the 26-week treatment period. Lower urine pH was evident during the 12-week recovery period, but was not significantly different compared to the controls. Thus, lower urine pH was treatment-related.

Gross Pathology

All animals including unscheduled sacrifices and euthanized animals were subjected to a complete necropsy and gross examination. There were no treatment-related macroscopic findings, except for thickened subcutaneous injection sites in the groups treated with 8 or 25 mg/kg/dose.

Organ Weights

The following organs were weighed: adrenal, brain, epididymis, heart, kidney, liver, lung, ovary, pituitary gland, prostate, salivary gland (mandibular (2)), spleen, testis, thymus, thyroid with parathyroid, and uterus

At the end of the initial 17-week treatment period, statistically significant decreases in mean absolute weight and organ/brain weight ratio were noted in the liver (↓approximately 14%) and thyroid gland (↓22%) in males given 25 mg/kg/dose. At the end of the 4-week recovery period, statistically significant increases in mean absolute weight and organ/brain weight ratio were noted in the spleen (↑approximately 21%) and adrenal gland (↑approximately 26%) in females given 25 mg/kg/dose.

At the end of the 26-week treatment period, an increase in relative kidney weight (to bodyweight or to brain weight) was observed in all treated males, and a statistically significant increase was observed in the 1 and 25 mg/kg/dose groups (relative to body weight: ↑approximately 14%) and in the 1 and 8 mg/kg/dose groups (relative to brain weight: ↑13-15%). The increase was not dose-dependent. At the end of the 12-week recovery period, females in the 25 mg/kg/dose group had a 19% decrease in absolute spleen weight ($P \leq 0.05$).

Histopathology

Adequate Battery: yes

Peer Review: yes

Preserved tissues were examined microscopically from all animals in the control and treatment groups (including those found dead or sacrificed prior to scheduled necropsy). The following organs from each animal were preserved in 10% neutral buffered formalin, unless otherwise indicated below (a: Preserved in modified Davidson's fixative): adrenal, aorta, brain, cecum, cervix, colon, duodenum, epididymis[a], esophagus, eyes[a], femur with bone marrow (articular surface of the distal end), heart, ileum, injection site(s), jejunum, kidney, lesions, liver, lung with large bronchi, lymph node (mandibular and mesenteric), mammary gland (females), optic nerve[a], ovary, pancreas, pituitary gland, prostate, rectum, salivary gland (mandibular), sciatic nerve, seminal vesicle, skeletal muscle (thigh), skin/subcutis, spinal cord (cervical, thoracic, and lumbar), spleen, sternum with bone marrow, stomach, testis[a], thymus, thyroid with parathyroid, tongue, trachea, urinary bladder, uterus, and vagina.

Histological Findings:

At the end of the 17-week treatment period, drug-related histopathological findings were observed in the kidney, spleen, liver, testes, adrenal gland, mesenteric and mandibular lymph nodes, and subcutaneous injection sites as shown in the Sponsor's table below. Focal to multifocal areas of vacuolation/hypertrophy of renal tubule cells were observed in the kidney of males (3/10) in the 25 mg/kg/dose group and females in the 8 and 25 mg/kg/dose groups (1/10 and 2/10, respectively). Increased vacuolation, typically characterized by a single, clear cytoplasmic vacuole, was observed in histiocytic cells in the liver, spleen, mesenteric lymph node, and mandibular lymph node in males in the 25 mg/kg/dose group and females in the 8 and 25 mg/kg/dose groups and in the adrenal cortex of females in the 25 mg/kg/dose group. Small, micro-vacuolated histiocytic cells were also observed in the interstitium of the testes in the 25 mg/kg/dose group. At the

end of the 4-week recovery period, focal to multifocal areas of vacuolation/hypertrophy of renal tubule cells in the kidney (4/5) and vacuolation of histiocytic cells in the testes (3/5) persisted in males receiving 25 mg/kg/dose. Test article-related microscopic findings at subcutaneous injection sites included minimal to marked infiltrates of vacuolated histiocytic cells and lymphocytes, fibrosis, minimal degeneration of skeletal muscle and/or adipocytes.

Similar histopathological changes with increased incidence and severity were also observed in these organs at the end of the 26-week treatment period. The severity and incidence of treatment-related histopathological changes are summarized in the Sponsor's table below.

Table 31: Incidence and Severity of Test Article-Related Microscopic Findings at 17-Week Interim Phase Euthanasia in a 26-Week Rat Toxicity Study

Sex	Males				Females			
Dose Level rAvPAL-PEG (mg/kg/dose)	0	1	8	25	0	1	8	25
No. Examined	10	10	10	10	10	10	10	10
Kidney								
Vacuolation/Hypertrophy, Tubule Cell, Focal/Multifocal								
Minimal	0	0	0	3	0	0	1	2
Liver								
Vacuolation, Histiocytic Cells								
Minimal	0	0	0	5	0	0	1	6
Slight	0	0	0	0	0	0	0	2
Spleen								
Vacuolation, Histiocytic Cells								
Minimal	0	0	0	5	0	0	1	4
Slight	0	0	0	0	0	0	0	3
Moderate	0	0	0	0	0	0	0	3
Lymph Node, Mesenteric								
Vacuolation, Histiocytic Cells								
Minimal	0	0	0	5	0	0	2	7
Slight	0	0	0	0	0	0	0	3
Lymph Node, Mandibular								
Vacuolation, Histiocytic Cells								
Minimal	0	0	0	4	0	0	5	4
Slight	0	0	0	3	0	0	0	5
Adrenal, Cortex								
Vacuolation, Histiocytic Cells								
Minimal	0	0	0	0	0	0	0	3

Table 32: Incidence and Severity of Test Article-Related Microscopic Findings at 17-Week Interim Phase Euthanasia in a 26-Week Rat Toxicity Study (Continued)

Sex		Males				Females			
Dose Level rAvPAL-PEG (mg/kg/dose)	0	1	8	25	0	1	8	25
No. Examined	10	10	10	10	10	10	10	10
Testes								
Vacuolation, Histiocytic Cells								
Minimal	0	0	0	10	NA	NA	NA	NA
Subcutaneous Injection Site A								
Infiltrate, Histiocytic Cells, Vacuolated								
Minimal	0	4	0	0	0	3	4	0
Slight	0	1	7	0	0	0	5	0
Moderate	0	0	3	8	0	0	0	6
Marked	0	0	0	2	0	0	0	4
Fibrosis								
Minimal	0	0	3	1	0	0	2	1
Slight	0	0	2	0	0	0	1	0
Moderate	0	0	1	9	0	0	0	9
Infiltrate, Lymphocytes								
Minimal	0	2	1	3	0	2	2	4
Slight	0	1	0	0	0	0	1	0
Degeneration, Skeletal Muscle/Adipocytes, Dermis								
Minimal	0	0	0	2	0	0	0	0
Subcutaneous Injection Site B								
Infiltrate, Histiocytic Cells, Vacuolated								
Minimal	0	0	8	4	0	0	4	2
Slight	0	0	0	2	0	0	0	4
Moderate	0	0	0	2	0	0	0	2

Table 33: Incidence and Severity of Test Article-Related Microscopic Findings at the End of the 26-week SC Rat Toxicity Study

Sex		Males				Females			
Dose Level rAvPAL-PEG (mg/kg/dose)	0	1	8	25	0	1	8	25
No. Examined	15	12	15	15	15	15	15	15
Kidney								
Vacuolation/Hypertrophy, Tubule Cell, Focal/Multifocal								
Not Present	15	12	7	4	15	15	14	8
Minimal	0	0	8	10	0	0	1	7
Slight	0	0	0	1	0	0	0	0
Liver								
Vacuolation, Histiocytic Cells								
Not Present	15	12	15	7	15	15	14	6
Minimal	0	0	0	8	0	0	1	7
Slight	0	0	0	0	0	0	0	2
Spleen								
Vacuolation, Histiocytic Cells								
Not Present	15	12	15	5	15	15	14	0
Minimal	0	0	0	7	0	0	1	9
Slight	0	0	0	3	0	0	0	5
Moderate	0	0	0	0	0	0	0	1
Lymph Node, Mesenteric								
Vacuolation, Histiocytic Cells								
Not Present	15	12	15	8	15	15	11	4
Minimal	0	0	0	7	0	0	4	11
Lymph Node, Mandibular								
Vacuolation, Histiocytic Cells								
Not Present	15	12	15	1	15	15	10	1
Minimal	0	0	0	10	0	0	5	11
Slight	0	0	0	3	0	0	0	3

Table 34: Incidence and Severity of Test Article-Related Microscopic Findings at the End of the 26-week SC Rat Toxicity Study (Continued)

Sex	Males				Females			
Dose Level rAvPAL-PEG (mg/kg/dose)	0	1	8	25	0	1	8	25
No. Examined	15	12	15	15	15	15	15	15
Testes								
Vacuolation, Histiocytic Cells								
Not Present	15	12	15	2	NA	NA	NA	NA
Minimal	0	0	0	13	NA	NA	NA	NA
Subcutaneous Injection Site A								
Infiltrate, Histiocytic Cells, Vacuolated								
Not Present	15	4	0	0	15	7	2	0
Minimal	0	6	1	0	0	8	1	0
Slight	0	2	3	1	0	0	6	2
Moderate	0	0	11	8	0	0	6	9
Marked	0	0	0	6	0	0	0	4
Fibrosis								
Not Present	15	7	1	1	15	15	2	0
Minimal	0	5	0	0	0	0	3	1
Slight	0	0	1	1	0	0	7	2
Moderate	0	0	13	13	0	0	3	12
Degeneration, Skeletal Muscle/Adipocytes, Dermis								
Not Present	15	12	13	2	15	15	13	1
Minimal	0	0	2	6	0	0	2	4
Slight	0	0	0	5	0	0	0	7
Moderate	0	0	0	2	0	0	0	3
Subcutaneous Injection Site B								
Infiltrate, Histiocytic Cells, Vacuolated								
Not Present	15	11	3	6	15	14	10	4
Minimal	0	1	12	8	0	1	5	10
Slight	0	0	0	1	0	0	0	1
Degeneration, Skeletal Muscle/Adipocytes, Dermis								
Not Present	15	12	13	13	15	15	13	9
Minimal	0	0	2	2	0	0	2	6

At the end of the 12-week recovery phase, focal and multi-focal vacuolation/ hypertrophy in kidney tubule cells and minimal to slight vacuolation in histiocytic cells persisted in organs (liver, spleen, adrenal, lymph nodes and testes), although these changes occurred with lower incidence and less severity. The injection sites had the same microscopic findings as that observed at the end of the 17-week treatment period, but with higher incidence and greater severity. At the end of the 12-week recovery period, infiltrate and vacuolation in histiocytic cells and fibrosis were still observed in injection site A, but with less severity. The severity and incidence of test article-related histopathological changes are summarized in the Sponsor's table below.

Table 35: Incidence and Severity of Test Article-Related Microscopic Findings at the End of the 12-Week Recovery Phase in a 26-Week SC Rat Toxicity Study

Sex	Males		Females	
Dose Level rAvPAL-PEG (mg/kg/dose)	0	25	0	25
No. Examined	5	5	5	5
Kidney				
Vacuolation/Hypertrophy, Tubule Cell, Focal/Multifocal				
Not Present	5	0	5	2
Minimal	0	4	0	3
Slight	0	1	0	0
Liver				
Vacuolation, Histiocytic Cells				
Not Present	5	5	5	4
Minimal	0	0	0	1
Spleen				
Vacuolation, Histiocytic Cells				
Not Present	5	2	5	0
Minimal	0	3	0	4
Slight	0	0	0	1
Lymph Node, Mesenteric				
Vacuolation, Histiocytic Cells				
Not Present	5	4	5	0
Minimal	0	1	0	5
Lymph Node, Mandibular				
Vacuolation, Histiocytic Cells				
Not Present	5	0	5	0
Minimal	0	5	0	5
Adrenal, Cortex				
Vacuolation, Histiocytic Cells				
Not Present	5	5	5	4
Minimal	0	0	0	1
Testes				
Vacuolation, Histiocytic Cells				
Not Present	5	1	NA	NA
Minimal	0	4	NA	NA
Subcutaneous Injection Site A				
Infiltrate, Histiocytic Cells, Vacuolated				
Not Present	5	1	5	3
Minimal	0	4	0	2
Fibrosis				
Not Present	5	1	5	2
Minimal	0	0	0	1
Slight	0	4	0	2

Toxicokinetics

Samples were collected pre-dose and at approximately 8, 24, 48, 72, 78, 96, 120, 144, 168, 192, 216, 240, and 264 hours post-dose on days 1, 22, 50, 78, 109, 141, 183 and 186. Plasma levels of rAvPAL-PEG were determined using a sandwich ELISA. The lower limit of quantification (LLOQ) for this assay in rat serum was 80 ng/mL.

Overall, systemic exposure of rAvPAL-PEG ($AUC_{0\text{-}72hr}$ and C_{max}) appeared proportional with doses of 1 to 25 mg/kg rAvPAL-PEG over weeks 1, 8, 16 and 27. Overall, systemic exposure to rAvPAL-PEG was greater in females than males. The decline in systemic levels after the first week may have been due to the formation of antibodies against rAvPAL-PEG. There was almost no accumulation of rAvPAL-PEG with repeated doses. The TK parameters are summarized in the tables below (taken from the study report).

Table 36: Toxicokinetic Parameters for rAvPAL-PEG in Rat Plasma (n = 3/sex) Following 17- and 26-Week Treatment Periods

Group No.	Dose Level mg/kg	Sex	Study Week	$AUC_{0\text{-}72\ hr}$ ng-hr/mL	C_{max} ng/mL	T_{max}[a] hr
6	1	Male	1	60406	1299	48
			8	8188	552	0
			16	16372	499	0
			27	10064	315	24
		Female	1	75652	1352	24
			8	0	0	NA
			16	114660	5671	72
			27	124876	3673	72
		Combined	1	68029	1325	36
			8	4094	276	0
			16	65516	3085	36
			27	67470	1994	48
7	8	Male	1	970572	26775	48
			8	511604	12817	24
			16	429352	10625	72
			27	489192	11242	8
		Female	1	1917172	49483	48
			8	2722848	75867	8
			16	1768028	34267	48
			27	1584332	25758	8
		Combined	1	1443872	38129	48
			8	1617226	44342	16
			16	1098690	22446	60
			27	1036762	18500	8
8	25	Male	1	2983308	62633	72
			8	6394576	126767	0
			16	3997860	68383	24
			27	3815748	69017	48
		Female	1	8122412	194717	48
			8	13324672	284233	8
			16	5566084	145583	0
			27	8750660	236870	24
		Combined	1	5552860	128675	60
			8	9859624	205500	4
			16	4781972	106983	12
			27	6283204	152944	36

NA = Is not applicable when the C_{max} value is 0.
a Collection times for Weeks 1, 8, 16 and 27 were predose (0), 8, 24, 48 and 72 hr.

Special Evaluation

1. Electron microscopic (EM) examination

Cortex and medulla of the kidney from all animals were collected and embedded in epoxy resin for possible future processing and examination. No data was submitted in this study report.

2. Antibody analysis

Blood samples were collected from non-fasted animals once before initiation of dosing and during the treatment period (days 15, 29, 56, 92, 120, and 186), and recovery period (days 134, 148, 200, 211, and 246). Blood was also collected from animals euthanized at an unscheduled interval.

Anti-rAvPAL IgG titers were measurable by day 15 or 29 in most animals. A total of three males and four females in the control group had either a single time-point, two consecutive time-points, or more than two consecutive time-points with measurable antibody titers during the study. One male and two females in the 8 mg/kg/dose group had no measurable anti-rAvPAL IgG titers through day 120. Overall, animals treated in the 1 mg/kg/dose group had the highest titers and animals treated with 25 mg/kg/dose rAvPAL-PEG had the lowest titers.

One male in the interim dosing phase and one female in the final dosing phase in the 1 mg/kg/dose group were positive for neutralizing antibodies to rAvPAL on day 120. All other animals were negative for anti-rAvPAL neutralizing antibodies throughout the study.

One male and two females in the 8 mg/kg/dose group had no measurable anti-rAvPAL IgG titers through day 120 or day 186, respectively. Two males (one in the 25 mg/kg/dose group and one in the 8 mg/kg/dose group) and one female in the 8 mg/kg/dose group had measurable antibody titers at some time-points during the study, but had no measurable titers immediately prior to scheduled termination. The incidence of anti-drug antibodies in the study animals is presented in the Sponsor's table below.

Table 37: Incidence of Antibody Positive Animals in a 26-Week SC Rat Toxicity Study

		Incidence of Antibody Positive Animals following twice weekly SC dosing with rAvPAL-PEG				
		Anti-rAvPAL IgG				Anti-rAvPAL-PEG NAb
Group	Dose, mg/kg	No. Positive at baseline	No. Positive during dosing phase of study	No. Positive at end of Interim Recovery Euthanasia	No. positive at end of Final Recovery Euthanasia	No. positive
1	Control	1M/0F	3M[a]/4F[a]	0M/0F	0M/0F	0M/0F
2	1	0M/0F	25M/25F[b]	-	-	1M[e]/1F[e]
3	8	0M/0F	24M[c]/23F[c]	-	-	0M/0F
4	25	0M/0F	35M[d]/35F	5M/5F	5M/5F	0M/0F

- Not applicable
a: Control animals had either a one to three consecutive ant body-positive samples, with the exception of one female (Animal No. 77933) that had five consecutive ant body-positive samples from Day 15 to Day 120.
b: One female (Animal No. B77968) had only two positive ant body samples (Day 120 and 186).
c: One male (Animal No. B77830) and one female (Animal No. B77994) had only one positive antibody sample (Day 56 and 92, respectively). One female (Animal No. B77980) had only two consecutive positive ant body samples (Day 92 and 120).
d: One male (Animal No. B77873) had only one positive antibody sample during the dosing phase (Day 120) and three positive samples during the recovery phase (Day 200, 211 and 272). One male (Animal No. B77856) had only two positive antibody sample (Day 92 and 186). One male (Animal No. B77850) had only one positive antibody sample (Day 92).
e: One male (Animal No. B77797) and one female (Animal No. B77958) were positive for neutralizing antibodies on Day 120.

3. Immunohistochemistry

The kidney from all animals in the control, treatment and recovery groups, and the liver from the recovery group animals were processed for immunohistochemistry. However, only kidney and liver tissue from four animals (one male and one female from the control group and one male and one female from the high-dose group) were subjected to immunohistochemistry examination in a feasibility study. In this study, tissue sections were stained with rabbit IgG anti-rAvPAL-PEG (also called BP84ex) or rabbit anti-PEG. There was no staining in the tissues from the control group.

In tissues from the 25 mg/kg/dose group, staining with IgG anti-rAvPAL-PEG was associated with intravascular and/or interstitial proteinaceous material in endothelium, arterial intima, and liver mononuclear cells and Kupffer cells. Staining with anti-PEG antibodies was observed in liver Kupffer cells. These locations suggested intravascular uptake of rAvPAL-PEG, possible phagocytosis by resident phagocytic cells, and systemic distribution following subcutaneous injection. Vacuolated histiocytes in liver and glomerular mesangium and vacuolated tubule cells in kidney were positive for staining with anti-PEG antibodies.

Furthermore, semi-quantitative analysis of PEG accumulation in adrenal gland, kidney, spleen, liver, lymph nodes (mesenteric and mandibular), and testis was conducted using a validated immunohistochemistry method. The study report (16-RS-357) is reviewed in the Special Toxicology Studies section of this review.

Dosing Solution Analysis

Duplicate samples were collected for homogeneity analysis from the top, middle, and bottom of the 0.4 and 10 mg/ml formulations for days 1. The three sampling regions in the 0.4 mg/mL dose solution were at the nominal concentration, indicating that it was a completely homogenous solution. However, the homogeneity for the 10 mg/mL dose solution was lower by an average of 10% relative to the intended concentration. The Sponsor stated the following: *"Because these results were similar to those obtained from past studies with this protein, the formulations were considered homogenous, and the animals received the intended doses of the test article."*

The stability of the 0.4 and 10 mg/ml formulations for days 1 and 179 was analyzed at time zero and at the end of dosing on days 1 and 179 of the dosing phase. There was no noticeable change in activity for either 0.4 or 10 mg/mL formulation between time zero and the end of dosing on days 1 and 179. The dose solutions were considered to be stable.

Study title: 4-Week Subcutaneous Injection Toxicity and Toxicokinetic Study with rAvPAL-PEG in Cynomolgus Monkeys with a 4-Week Recovery Phase

Study no.:	0165-07-008
Study report location:	N/A
Conducting laboratory and location:	(b) (4)
Date of study initiation:	April 19, 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	NP1060701, Purity: 99.2%,

Key Study Findings

1. Treatment-related histopathological changes included:
   - Perivascular infiltrates of lymphocytes and macrophages, and granulomatous inflammation in the subcutis of injection sites at all doses.
   - Degeneration of blood vessels (predominantly medium-sized muscular arteries) in the following organs at 0.1 or 1 mg/kg: lung, gallbladder, kidney, colon, pancreas, spleen, stomach, and prostate.
2. rAvPAL-PEG at 0.1 and 1 mg/kg decreased plasma phenylalanine.
3. The target organs of toxicity were arteries and injection sites.

4. The NOAEL was 0.01 mg/kg, based on degeneration of arteries at 0.1 mg/kg and higher.

Methods

Doses:	0 (vehicle), 0.01, 0.1, and 1 mg/kg
Frequency of dosing:	Twice weekly
Route of administration:	The first 7 subcutaneous injections were dosed at the same site, while the eighth dose was injected on the contralateral side. The locations of injection sites were not described in the study report.
Dose volume:	0.5 mL/kg
Formulation/Vehicle:	rAvPAL-PEG was dissolved in control article/diluent (10 mM tris (hydroxymethyl) aminomethane (TRIS) and 140 mM sodium chloride (NaCl) at concentrations of 0.02, 0.2, and 2 mg/ml.
Species/Strain:	cynomolgus monkeys (*Macaca fascicularis*)
Number/Sex/Group:	3-5/sex/group
Age:	2 to 3 years old
Weight:	Males: 2.5 - 3 kg Females: 2.2 - 2.4 kg
Satellite groups:	2/sex/group for recovery groups
Unique study design:	N/A
Deviation from study protocol:	None of the deviations had impacts on integrity or interpretability of the results.

Observations and Results

Mortality

Animals were checked twice daily for mortality. There were no deaths.

Clinical Signs

Clinical signs were monitored twice daily. There were no treatment-related clinical signs.

Body Weights

Body weight was recorded 4 times during the pre-dose phase, before injection on each day of dosing, and weekly thereafter. There were no treatment-related changes in body weight or body weight gain.

Feed Consumption

Qualitative food consumption was assessed once daily. There were no treatment-related changes in food consumption.

Ophthalmoscopy

Ophthalmic examination was performed during the pre-dose phase and during the final week of the dosing phase. There were no treatment-related findings.

ECG

ECG was recorded once during the pre-dose phase and during the final week of the dosing phase. There were no treatment-related findings.

Hematology

Blood samples were collected from fasted animals prior to treatment initiation and during the treatment (days 3 and 23) and recovery periods (day 28). There were no treatment-related changes.

Clinical Chemistry

Blood samples were collected from fasted animals prior to treatment initiation and during the treatment (days 3, and 23) and recovery periods (day 28). There were no treatment-related findings.

Urinalysis

Urine samples were collected from fasted animals prior to treatment initiation and during the treatment (days 3, and 23) and recovery periods (day 28). rAvPAL-PEG had no adverse effects on urinalysis parameters (e.g. urine volume, specificity, pH, protein, glucose).

Gross Pathology

At termination, all animals were subjected to necropsy. Terminal body weights were recorded.

There were no treatment-related macroscopic changes at the end of the treatment and recovery periods.

Organ Weights

At scheduled termination, the following organs (when present) were weighed (paired organs were weighed together): adrenal, brain, epididymis, heart, kidney, liver with gallbladder, lung, ovary, pituitary gland, prostate, salivary gland (mandibular), seminal vesicle, spleen, testis, thymus, thyroid with parathyroid, and uterus. There were no treatment-related changes in absolute and relative organ weights.

Histopathology

Adequate Battery: Yes

Peer Review: Yes

The following tissues from all animals were preserved in 10% neutral-buffered formalin with the exception of the eyes, optic nerves, and testes, which were preserved in modified Davidson's fixative: adrenal glands, aorta (thoracic), brain, cecum, cervix, colon, duodenum, epididymides, esophagus, eyes, femur with bone marrow (articular surface of the distal end) gallbladder, heart, ileum, injection site(s), jejunum, kidneys, lacrimal gland, liver, lung with large bronchi, cervical lymph node, mesenteric lymph node, mammary gland (region), skeletal muscle (thigh), optic nerves, ovaries, pancreas, parathyroid, pituitary, prostate, rectum, mandibular salivary gland, sciatic nerve, seminal vesicles, skin, spinal cord (cervical and thoracic), spleen, sternum with bone marrow, stomach, testes, thymus, thyroids, tongue, trachea, urinary bladder, uterus, vagina, and any gross lesions. All tissues as listed above were microscopically examined.

Histological Findings

Treatment-related histopathological changes were observed in arteries, injection sites, and liver.

Kupffer cell hypertrophy/hyperplasia (minimal to slight) in the liver was observed in treated animals. There were no-dose dependent effects.

The incidence of finely granular brown intracytoplasmic pigment in proximal tubular epithelial cells (minimal to slight) in kidney did not appear to be treatment related, based on the totality of the data from the main study and recovery groups.

Males in the 0.1 and 1 mg/kg groups and 1 female in the 1 mg/kg group had minimal to slight degeneration of blood vessels in some organs (lungs, gallbladder, kidney, colon, pancreas, spleen, and prostate in the males treated with 0.1 mg/kg, lungs in the male given 1 mg/kg, and lungs and stomach in the female given 1 mg/kg). The changes were characterized by basophilia, vacuolation, and small amounts of cellular debris and/or infiltrates of foamy macrophages within the tunica intima and/or tunica media of predominantly medium-sized muscular arteries.

The incidence and severity of treatment-related histopathological changes in liver, kidney, and injection sites are summarized in the table below (taken from the study report).

Table 38: Incidence and Severity of Selected Microscopic Findings – Main Study

	rAvPAL-PEG							
Sex	Males				Females			
Group	1	2	3	4	1	2	3	4
Dose Level (mg/kg/dose)	0	0.01	0.1	1	0	0.01	0.1	1
No. Examined	3	3	3	3	3	3	3	3
Subcutaneous Site A								
Infiltrate, Lymphocytes/Macrophages, Perivascular, Subcutis								
Not Present	3	0	0	0	2	0	0	0
Minimal	0	2	2	2	1	2	1	1
Slight	0	1	1	1	0	1	1	2
Moderate	0	0	0	0	0	0	1	0
Inflammation, Granulomatous, Subcutis								
Not Present	3	2	1	3	1	1	2	2
Minimal	0	0	2	0	2	2	1	1
Slight	0	1	0	0	0	0	0	0
Subcutaneous Site B								
Infiltrate, Lymphocytes/Macrophages, Perivascular, Subcutis								
Not Present	3	0	0	1	2	0	0	0
Minimal	0	3	3	0	1	3	2	1
Slight	0	0	0	2	0	0	1	1
Moderate	0	0	0	0	0	0	0	1
Inflammation, Granulomatous, Subcutis								
Not Present	2	1	3	3	3	2	1	2
Minimal	1	2	0	0	0	1	2	0
Slight	0	0	0	0	0	0	0	1
Kidney								
Pigment, Tubule Cell								
Not Present	3	2	3	1	3	3	2	3
Minimal	0	1	0	1	0	0	1	0
Slight	0	0	0	1	0	0	0	0
Liver								
Hypertrophy/Hyperplasia, Kupffer Cell								
Not Present	3	3	1	2	3	2	0	3
Minimal	0	0	2	0	0	1	2	0
Slight	0	0	0	1	0	0	1	0

At the end of the recovery period, the changes in the injection sites (minimal perivascular infiltrates of lymphocytes and macrophages and minimal granulomatous inflammation in the subcutis) were not completely reversed. Vascular lesions were not observed in any animal at the recovery phase necropsy.

Table 39: Incidence and Severity of Selected Microscopic Findings - Recovery Phase

	rAvPAL-PEG			
	Males		Females	
Group	1	4	1	4
Dose Level (mg/kg/dose)	0	1	0	1
No. Examined	2	2	2	2
Subcutaneous Site A				
Infiltrate, Lymphocytes/Macrophages, Perivascular, Subcutis				
Not Present	2	1	2	2
Minimal	0	1	0	0
Inflammation, Granulomatous, Subcutis				
Not Present	1	2	2	2
Minimal	1	0	0	0
Subcutaneous Site B				
Infiltrate, Lymphocytes/Macrophages, Perivascular, Subcutis				
Not Present	2	1	2	2
Minimal	0	1	0	0
Inflammation, Granulomatous, Subcutis				
Not Present	2	1	2	2
Minimal	0	1	0	0
Kidney				
Pigment, Tubule Cell				
Not Present	2	1	2	1
Minimal	0	1	0	1
Liver				
Hypertrophy/Hyperplasia, Kupffer Cell				
Not Present	1	1	0	2
Minimal	1	1	2	0

Toxicokinetics

Blood samples were collected from non-fasted animals on days 1, 8, 22, and 25 of the dosing phase, using collection times of 0 (pre-dose), 9, 24, 36, 48, 60, and 72 hr post-dose. After the last dose administration (dose 8 on day 25), blood samples were collected from all animals at 24 hr post-dose, and blood samples from all animals in the recovery groups were continually collected at 48, 72, 96, 120, 144, 168, 216, 264, 312, 360, 504, and 672 hr post-dose. Plasma rAvPAL-PEG levels were measured using an ELISA method. The lower limit of quantitation (LLOQ) for this assay was 100 ng/ml. All plasma drug concentrations in the 0.01 mg/kg males were below the lower limit of quantitation after doses 1 and 3.

Because of the high variation in plasma concentration profiles in individual samples, the TK data should be interpreted with caution.

Exposure to rAvPAL-PEG generally increased with dose. After three doses, rAvPAL-PEG accumulated in plasma. rAvPAL-PEG exhibited slow absorption and slow elimination from plasma. In general, females had higher mean rAvPAL-PEG C_{max} and AUC_{0-t} values compared to males. The TK data is summarized in the tables below (taken from the study report).

Table 40: Mean Toxicokinetic Parameters for rAvPAL-PEG in Monkey Plasma - Dose 1

Dose Group	Dose Level (mg/kg/dose)	Sex		C_{max} (ng/mL)	DN C_{max} [(ng/mL) / (mg/kg/dose)]	T_{max} (hr)	AUC_{0-t} (ng•hr/mL)	DN AUC_{0-t} [(ng•hr/mL) / (mg/kg/dose)]
2	0.01	M		NA	NA	NA	NA	NA
		F	Mean	128	12750	42.0	2318	231750
			SD	NA	NA	NA	NA	NA
			N	2	2	2	1	1
3	0.1	M	Mean	573	5733	40.0	27283	272825
			SD	331	3308	6.9	19308	193079
			N	3	3	3	3	3
		F	Mean	805	8050	26.0	32850	328500
			SD	469	4687	29.4	11270	112699
			N	3	3	3	3	3
	Combined		Mean	689	6892	33.0	30066	300663
			SD	384	3844	20.6	14465	144645
			N	6	6	6	6	6
4	1	M	Mean	9168	9168	37.8	447867	447867
			SD	4664	4664	16.9	200984	200984
			N	5	5	5	5	5
		F	Mean	10578	10578	45.6	402864	402864
			SD	6476	6476	10.0	172912	172912
			N	5	5	5	5	5
	Combined		Mean	9873	9873	41.7	425366	425366
			SD	5372	5372	13.7	178337	178337
			N	10	10	10	10	10

Note: The combined is the mean of the males and females.

Table 41: Mean Toxicokinetic Parameters for rAvPAL-PEG in Monkey Plasma - Dose 3

Dose Group	Dose Level (mg/kg/dose)	Sex		C_{max} (ng/mL)	DN C_{max} [(ng/mL) / (mg/kg/dose)]	T_{max} (hr)	AUC_{0-t} (ng•hr/mL)	DN AUC_{0-t} [(ng•hr/mL) / (mg/kg/dose)]	C_{max} Accumulation	AUC_{0-t} Accumulation
2	0.01	M	Mean	NA	NA	NA	NA	NA	NA	NA
		F	Mean	280	28000	36.0	7890	789000	2.15	NA
			SD	NA	NA	NA	NA	NA	NA	NA
			N	1	1	1	1	1	1	0
3	0.1	M	Mean	1588	15883	44.0	90318	903175	3.45	4.96
			SD	778	7776	6.9	42579	425791	2.10	8.15
			N	3	3	3	3	3	3	3
		F	Mean	570	5700	9.00	39679	396788	1.05	1.54
			SD	406	4061	0	NA	NA	0.88	NA
			N	3	3	3	2	2	3	2
	Combined		Mean	1079	10792	26.5	70062	700620	2.25	3.59
			SD	787	7867	19.7	40973	409733	1.95	6.07
			N	6	6	6	5	5	6	5
4	1	M	Mean	23947	23947	30.6	1185270	1185270	2.74	2.82
			SD	13931	13931	28.2	409625	409625	1.30	0.69
			N	5	5	5	5	5	5	5
		F	Mean	31615	31615	48.0	1292579	1292579	3.57	3.82
			SD	10181	10181	30.6	425589	425589	1.61	2.27
			N	5	5	5	5	5	5	5
	Combined		Mean	27781	27781	39.3	1238924	1238924	3.16	3.32
			SD	12193	12193	29.2	397836	397836	1.45	1.67
			N	10	10	10	10	10	10	10

Note: The combined is the mean of the males and females.

Table 42: Dose Proportionality Ratios for rAvPAL-PEG in Monkey Plasma

		Males		Females	
Interval	Actual Dose Level Increase	C_{max} Dose Ratio[a]	AUC_{0-t} Dose Ratio[a]	C_{max} Dose Ratio	AUC_{0-t} Dose Ratio
Dose 1	1.0:10:100 - fold	1.0:16 - fold	1.0:16 - fold	1.0:6.3:83 - fold	1.0:14:174 - fold
Dose 3	1.0:10:100 - fold	1.0:15 - fold	1.0:13 - fold	1.0:2.0:113 - fold	1.0:5.0:164 - fold

a Comparison limited to Dose Groups 3 (0.1 mg/kg) and 4 (1 mg/kg).

Special Evaluation

1. Electron Microscopic Examination

Kidney samples from males and females in the control and 7/5/3 mg/kg/ dose groups were collected at termination of the end treatment and recovery periods. However, only renal cortex was examined to characterize morphologic effects in renal cortex glomeruli and proximal convoluted tubule epithelium. There were no treatment-related ultrastructural changes in the renal cortex (i.e. primarily glomerulus and proximal convoluted tubule epithelium).

2. Antibody Analysis

Blood samples were collected from non-fasted animals prior to treatment initiation and during the treatment (days 10, 14, 22, and 28) and recovery periods (every 2 weeks).

Antibody determinations were conducted for anti-rAvPAL-PEG IgG and IgM antibodies using a validated sandwich enzyme linked immunosorbent assay (ELISA) method.

By days 22 and 28 of the dosing phase, all animals in the 1 mg/kg group were positive for anti-rAvPAL-PEG IgG antibodies, with measurable titers persisting through the end of the recovery period. The number of animals that tested positive for anti-rAvPAL-PEG IgG antibodies increased with treatment duration. However, no IgM rAvPAL-PEG antibodies were detected during the course of the study.

3. Phenylalanine Analysis

Blood samples were collected from non-fasted animals prior to treatment initiation (day 13), and during the treatment (pre-dose, and 9, 24, 48, 72 hr post-dose on a weekly basis) and recovery periods (twice weekly). Blood was collected in conjunction with toxicokinetic and/or clinical pathology collections once during the pre-dose phase (day 13), and in conjunction with the toxicokinetic sampling times throughout the remainder of the dosing and recovery phases as specified in the toxicokinetics section, and at the scheduled sacrifices. Phenylalanine in plasma was analyzed using LC-API/MS/MS detection. Phenylalanine-ring-$^{13}C6$ was used as the internal standard. The lower limit of quantitation (LLOQ) was established at 1 µmol/L.

A single dose of 0.1 or 1 mg/kg rAvPAL-PEG produced a moderate to marked decrease in plasma phenylalanine levels that occurred within 9 hr and 22 hr post-dose for 1 mg/kg and 0.1 mg/kg rAvPAL-PEG, respectively. The marked decrease in plasma phenylalanine remained through days 11 and 22 for the 0.1 mg/kg and 1 mg/kg groups, respectively, following repeated administration of rAvPAL-PEG. The plasma phenylalanine levels were comparable to that of control animals during the rest of treatment and the recovery periods. rAvPAL-PEG at 0.01 mg/kg/dose had no effect on plasma phenylalanine.

4. Amino Acid Analysis of Cerebrospinal Fluid

CSF (approximately 0.6 mL) samples were collected prior to scheduled sacrifices from the cisternae magna (accessed from the back of the neck) and were stored frozen for future analysis.

Dosing Solution Analysis

All dose formulations were stored at room temperature within 3 hours of preparation (dilution of test article), prior to dosing. The dose solutions of 0.02 and 2 mg/ml were stable from time 0 to 3 hr post-preparation. The 2 mg/ml formulation was homogeneous, and the drug concentration was within 10% of the nominal concentration. However, the top sample for the 0.02 mg/mL concentration was 50% higher than the theoretical value, while the middle and bottom samples matched nominal concentration. Therefore, the 0.02 mg/mL formulation was not homogeneous. The 0.02 mg/mL formulation was used in the low dose group, in which the animals received 0.02 mg/mL or higher. This group had no test article-related effects noted,

therefore the use of a non-homogeneous formulation was considered to have no significant impact on the study.

Study title: 39-Week Subcutaneous Injection Toxicity and Toxicokinetic Study with rAvPAL-PEG in Cynomolgus Monkeys with a 13-Week Recovery Phase

Study no.:	0165-07-030
Study report location:	N/A
Conducting laboratory and location:	(b) (4)
Date of study initiation:	November 14, 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	See table below

Table 43: Lot Numbers and Purities in a 39-Week SC Monkey Toxicity Study

Test Article	Lot No.	Storage	Purity	Expiration Date	Reserve (Archive) Sample
rAvPAL-PEG	NP160702	In a freezer, set to	100%	05 Jul 2008	Collected
	NP160706	maintain -60 to -80°C	100%	13 Sep 2008	Collected
	NP160801		100%	28 Feb 2009	Collected
	AP160703		100%	19 Jul 2008	Collected

Key Study Findings

In a 39-week toxicity study with a 13-week recovery period, male and female cynomolgus monkeys were administered rAvPAL-PEG by subcutaneous injection, twice weekly, at initial dose levels of 0.01, 0.1, 1, 3, and 7 mg/kg/dose. Females did not tolerate the 7 and 5 mg/kg dose levels, based on body weight loss, decreased food consumption, hypoactivity, anorexia, hematology and clinical chemistry changes, and poor clinical condition. The dose for both males and females was further reduced to 3 mg/kg/dose.

The major treatment-related microscopic finding was arterial inflammation in multiple organs and tissues in the 3 and 7/5/3 mg/kg/dose groups. The affected organs included kidney, urinary bladder, pancreas, gallbladder, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, lung, heart, sciatic nerve, lacrimal gland, mandibular lymph node, epididymis, seminal vesicle, ovary, uterus, cervix, and vagina.

Increased lymphoid nodules in bone marrow of the sternum occurred at 3 and 7/5/3 mg/kg/dose, and this change was not completely reversible. Immune-complex components presented in affected vessels in the heart, kidney, and liver in animals treated with 3 or 7/5/3 mg/kg/dose. In groups treated with 3 mg/kg/dose or higher,

rAvPAL-PEG and/or PEG were detected in intravascular proteinaceous material, endothelium, interstitial proteinaceous material, and mononuclear cells in the liver and kidney. The test article was also detected in liver Kupffer cells, and in a few hepatocytes in the 7/5/3 mg/kg/dose group. Perivascular cellular infiltrates were observed in subcutaneous injection sites in all treatment groups.

The target organs of toxicity were arteries in multiple organs, and bone marrow. The NOAEL was considered to be 1 mg/kg/dose due to arterial inflammation in multiple organs, deposition of immune-complex components in inflamed vessels of the heart, kidney, liver, and lymphoid nodules in bone marrow, at 3 and 7/5/3 mg/kg/dose.

Methods

Doses:	0 (vehicle), 0.01, 0.1, 1, 3, 7/5/3 mg/kg
Frequency of dosing:	Twice weekly
Route of administration:	Subcutaneous injection (injections into the dorsal region was rotated between three dose sites (A, B, and C), except for the final dose, which was injected into site D).
Dose volume:	1 mL/kg
Formulation/Vehicle:	rAvPAL-PEG was dissolved in control article/diluent (10 mM tris (hydroxymethyl) aminomethane (TRIS), 135 mM sodium chloride (NaCl), and 1 mM trans-cinnamic acid (tCA), 1 mM ammonia (NH3)) at concentrations of 0.01, 0.1, 1, 3, 5, and 7 mg/ml.
Species/Strain:	cynomolgus monkeys (*Macaca fascicularis*)
Number/Sex/Group:	3-8/sex/group
Age:	3 to 6 years old
Weight:	Males: 3.2 to 4.8 kg Females: 2.6 to 4.5 kg
Satellite groups:	3/sex/group for recovery groups
Unique study design:	N/A
Deviation from study protocol:	None of the deviations had impacts on integrity or interpretability of the results.

Study design is summarized in the table below (taken from study report).

Table 44: Study Design of a 39-Week SC Monkey Toxicity Study

Group[a]	No. of Animals[b] Male	Female	Dose Level (mg/kg/dose)	Dose Concentration (mg/mL)
1 (Control)	8	8	0	0
2	3	3	0.01	0.01
3	3	3	0.1	0.1
4	3	3	1.0	1.0
5	7	7	3.0	3.0
6	8	8	7.0/5.0/3.0[c]	7.0/5.0/3.0[c]

a Group 1 received control article only.
b Animals designated for recovery phase sacrifice (three animals/sex in Groups 1, 5, and 6) underwent 13 weeks of recovery following dose administration.
c Group 6 animals were administered 7.0 mg/kg/dose on Days 1, 4, and 8; were not dosed on Days 11 and 15; were administered 5.0 mg/kg/dose on Day 18; were not dosed on Days 22 through 43; and resumed twice weekly dosing on Day 46 at 3.0 mg/kg/dose.

Observations and Results

Mortality

Animals were checked twice daily for mortality. A female receiving 7 mg/kg/dose was euthanized on day 12 due to test article-related anorexia, hematology and clinical chemistry changes (decreases in red blood cell count, hemoglobin, hematocrit, absolute reticulocyte count, and total protein) and poor clinical condition. Alopecia of the skin, discoloration of the stomach, enlarged thyroids, and moderate atrophy of the thymus were observed. The cause of morbidity remained unclear.

Clinical Signs

Clinical signs were monitored twice daily. Due to adverse clinical signs (e.g. reduced or no food consumption, body weight loss, and hypoactivity), the 7 mg/kg/dose in group 6 was reduced to 5 mg/kg/dose, and then to 3 mg/kg/dose (see study design table above for details about changes in the dosing of group 6). No treatment-related clinical signs were observed in animals treated with up to 3 mg/kg/dose. Lactated Ringer's solution was administered subcutaneously to the group 6 females on a daily basis at a minimum dose of 100 mL on days 12 through 17 of the dosing phase to prevent dehydration.

Body Weights

Body weight was recorded at least once during the pre-dose phase, before dosing on day 1 of the main study, and weekly thereafter.

Overall, the mean body weights in males in all treatment groups were comparable to the controls during the treatment period (see table below). The high-dose (7 mg/kg/dose) females had a slight weight loss (0.1 kg) during the first week of treatment. The loss of bodyweight was associated with poor food consumption and hypoactivity. Otherwise, the mean body weights in females in all treatment groups were comparable to the controls. At the end of the recovery period, the body weight in the high-dose males was 6% higher than the controls.

Table 45: Body Weight in Male Monkeys in a 39-Week SC Toxicity Study

	Dose Groups (mg/kg/dose)					
Treatment period (Days)	Body Weight in Males (kg)					
	0	0.01	0.1	1	3	7/5/3
1	4.1	4.1	4.2	4.1	4.1	4.1
22	4.2	4.3	4.2	4.1	4.2	4.2
29	4.3	4.3	4.2	4.2	4.2	4.1
36	4.3	4.4	4.3	4.2	4.3	4.3
246	5.8	5.6	5.9	5.6	6.2	5.7
274	5.9	5.6	6	5.5	6.3	5.8
Recovery Period (days)						
7	6.4	-	-	-	6.1	6.3
49	6.6	-	-	-	6.3	6.8
91	6.4	-	-	-	6.2	6.8

Table 46: Body Weight in Female Monkeys in a 39-Week SC Toxicity Study

	Dose Groups (mg/kg/dose)					
Treatment period (Days)	Body Weight in Females (kg)					
	0	0.01	0.1	1	3	7/5/3
1	3.3	3.3	3.2	3.4	3.3	3.2
8	3.5	3.4	3.2	3.5	3.3	3.1
15	3.4	3.4	3.1	3.5	3.2	3.2
246	3.9	3.8	3.8	4	3.8	3.8
274	3.7	3.7	3.7	4	3.8	3.8
Recovery Period (days)						
7	3.3	-	-	-	4	3.6
49	3.3	-	-	-	4.1	3.6
91	3.4	-	-	-	4.2	3.6

Feed Consumption

Qualitative food consumption was assessed once daily. Animals treated with 7 or 5 mg/kg/dose (group 6) had low or no food consumption. The reduced food consumption was not observed after reducing the dose in group 6 to 3 mg/kg/dose. However, summary data was not present in the study report.

Ophthalmoscopy

Ophthalmic examination was performed during the pre-dose phase, during the final week of the main study, and week 12 of the recovery phase. There were no treatment-related findings.

ECG

ECG was recorded once during the pre-dose phase, during the final week of the main study, and week 13 of the recovery phase. A heart rate correction for the QT interval (QT_c) was calculated using the Bazett method. rAvPAL-PEG had no effects on ECG parameters (heart rate, QRS, PR, RR, QT, and QT_c intervals).

Hematology

Blood samples were collected from non-fasted animals (scheduled and unscheduled sacrifices) at pre-treatment, during treatment (study weeks 2, 4, 8, 12, 16, 20, 25, 30, 34, and 38) and recovery periods (study weeks 43, 48, and 53).

Significant decreases in RBC (↓11-12%), hemoglobin (↓13-14%), hematocrit (↓12-14%), absolute and relative reticulocytes (↓approximately 80% on day 15 only) were observed in females receiving 7 mg/kg/dose on days 15 and 25. rAvPAL-PEG at 0.01, 0.1, 1, and 3 mg/kg/dose had no adverse effects on hematology or coagulation parameters during the treatment period. No changes were observed during the recovery period.

Clinical Chemistry

Blood samples were collected from non-fasted animals (scheduled and unscheduled sacrifices) at pre-treatment, during the treatment period (weeks 2, 4, 8, 12, 16, 20, 25, 30, 34, and 38) and recovery periods (study weeks 43, 48, and 53).

On day 15, females given 7 mg/kg/dose had mild to moderate decreases in urea nitrogen (↓26%; $p \leq 0.05$), total protein (due to decreased albumin and globulin, ↓12%; $p \leq 0.05$), and cholesterol (↓26%; $P > 0.05$). All changes were treatment-related.

Urinalysis

Urine samples were collected from non-fasted animals (scheduled and unscheduled sacrifices) at pre-treatment, during the treatment period (study weeks 2, 4, 8, 12, 16, 20, 25, 30, 34, and 38) and recovery periods (study weeks 43, 48, and 53). rAvPAL-PEG

had no adverse effects on urinalysis parameters (urine volume, specificity, pH, protein, glucose, and etc.).

Gross Pathology

All surviving animals were subjected to necropsy at study termination. Terminal body weights were recorded. There were no treatment-related macroscopic changes at the end of the main study or recovery periods.

Organ Weights

At scheduled termination, the following organs (when present) were weighed (paired organs were weighed together): adrenal, brain, epididymis, heart, kidney, liver with gallbladder, lung, ovary, pituitary gland, prostate, salivary gland (mandibular), seminal vesicle, spleen, testis, thymus, thyroid with parathyroid, and uterus.

At the end of the main study, organ weight increases were noted in the prostate, seminal vesicles, epididymis, and testes. Increased spleen weight occurred in males and females.

Increased absolute and relative spleen weights were observed in the high-dose males (absolute weight ↑18%, relative to body weight ↑36%, and relative to brain weight ↑ 31%) and in females in all dose groups (absolute weight ↑up to 43%, relative to body weight ↑up to 59%, and relative to brain weight ↑44%).

Males in all treatment groups had increases in absolute and relative epididymis weight (dose-dependent absolute weight ↑20-45% ($p>0.05$), relative to body weight ↑ up to 50%, and relative to brain weight ↑up to 62%), prostate (absolute weight ↑up to 93%, relative to body weight ↑ up to 78%, and relative to brain weight ↑ 2-fold), and testis weights (absolute weight ↑ up to 66%, relative to body weight ↑ 73%, and relative to brain weight ↑ 82%).

Absolute and relative seminal vesicle weights were increased in males given ≥ 1.0 mg/kg/dose (absolute weight ↑ up to 64%, relative to body weight ↑ up to 53%, and relative to brain weight ↑ 1.9-fold).

Absolute and relative thymus weight decreased in males (relative to body weight ↓up to 39% and 41% in the 3 and 7/5/3 mg/kg/dose groups, respectively; relative to brain weight ↓35% in the 7/5/3 mg/kg/dose group) and females (absolute weight ↓22%, relative to body weight ↓14%, and relative to brain weight ↓20% in the 7/5/3 mg/kg/dose group).

Absolute and relative liver weight increased in males given 3 mg/kg/dose (absolute weight ↑20%, and relative to brain weight ↑31%; $p < 0.05$).

At the end of the recovery period, animals in the 3 or 7/5/3 mg/kg/dose group still had increases (non-significant) in absolute and relative weights of the following organs:

seminal vesicles (↑up to 53%), testis (↑up to 24%), epididymis (↑up to 13%), spleen (female, ↑up 70%), and liver (female, ↑up 18%). The relationship between organ weight changes and test-article remains uncertain since no correlating macroscopic or microscopic changes were observed in these organs. Variable maturity among dose groups may have influenced absolute and relative male reproductive organ weights.

Histopathology

Adequate Battery: Yes

Peer Review: Yes

The renal cortex and medulla were collected from all animals and stored in McDowell-Trump fixative for electron microscopy.

The following tissues from all animals, including the animal sacrificed prematurely, were preserved in 10% neutral-buffered formalin with the exception of the eyes, optic nerves, and testes, which were preserved in modified Davidson's fixative: adrenal glands, aorta (thoracic), brain, cecum, cervix, colon, duodenum, epididymides, esophagus, eyes, femur with bone marrow (articular surface of the distal end), gallbladder, heart, ileum, injection site(s), jejunum, kidneys, lacrimal gland, liver, lung with large bronchi, lymph node (cervical and mesenteric), mammary gland (region), skeletal muscle (thigh), optic nerves, ovaries, pancreas, parathyroid, pituitary, prostate, rectum, salivary gland, sciatic nerve, seminal vesicles, skin, spinal cord (cervical and thoracic), spleen, sternum with bone marrow, stomach, testes, thymus, thyroids, tongue, trachea, urinary bladder, uterus, vagina, and any gross lesions. All tissues listed above were microscopically examined.

Histological Findings

The major treatment-related microscopic finding was arterial inflammation in multiple tissues and organs from animals given 3 or 7/5/3 mg/kg/dose (groups 5 and 6, respectively). Other drug-related changes included an increased incidence and severity of perivascular cellular infiltrates in subcutaneous injection sites (1 mg/kg/dose and higher), and increased lymphoid nodules in the bone marrow of the sternum (7/5/3 mg/kg/dose).

Arterial inflammation in animals given 3 or 7/5/3 mg/kg/dose was observed in the following organs or tissues: kidney, urinary bladder, pancreas, gallbladder, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, lung, heart, sciatic nerve, lacrimal gland, mandibular lymph node, epididymis, seminal vesicle, ovary, uterus, cervix, vagina, and subcutaneous injection sites C and D. In addition, one female given 0.1 mg/kg/dose (group 3) had minimal arterial inflammation in the stomach.

Arterial inflammation was characterized by expansion and/or destruction of the tunica adventitia, tunica media, and/or tunica intima of small to midsize arteries, as mediated by infiltrating cells that included varying proportions of macrophages, lymphocytes,

fibroblasts, neutrophils, eosinophils, and plasmacytes. The vascular inflammatory changes included occasional cellular debris, vacuolation, and/or proteinaceous material. Rare arteries contained luminal thrombi. In the stomach of a female given 0.1 mg/kg/dose, one small artery had small amounts of karyorrhectic debris and apoptotic cells within the tunica media. The presence of arterial inflammation in a wide spectrum of organs suggested systemic arteritis. The incidence and severity of arterial inflammation are summarized in the table below (taken from the study report).

Table 47: Arterial Inflammation in Multiple Tissues or Organs in a 39-Week SC Monkey Toxicity Study (Main Study Sacrifice[a])

Sex	Male						Female					
Group	1	2	3	4	5	6	1	2	3	4	5	6
No. Examined	5[b]	3	3	3	4[b]	5	5[b,c]	3	3	3	4	4
Kidney	0	0	0	0	1 (1.0)	1 (1.0)	0	0	0	0	2 (2.5)	2 (2.5)
Urinary Bladder	0	0	0	0	0	0	0	0	0	0	0	1 (3.0)
Pancreas	0	0	0	0	0	1 (1.0)	0	0	0	0	1 (3.0)	1 (1.0)
Gallbladder	0	0	0	0	0	1 (1.0)	0	0	0	0	1 (2.0)	0
Esophagus	0	0	0	0	0	0	0	0	0	0	1 (2.0)	0
Stomach	0	0	0	0	0	0	0	0	1 (1.0)	0	1 (3.0)	2 (1.5)
Duodenum	0	0	0	0	0	1 (1.0)	0	0	0	0	1 (2.0)	1 (1.0)
Jejunum	0	0	0	0	0	1 (1.0)	0	0	0	0	1 (2.0)	0
Ileum	0	0	0	0	0	0	0	0	0	0	1 (2.0)	1 (1.0)
Cecum	0	0	0	0	0	3 (1.0)	0	0	0	0	1 (4.0)	2 (2.0)
Colon	0	0	0	0	0	3 (1.0)	0	0	0	0	1 (3.0)	0
Rectum	0	0	0	0	0	2 (1.0)	0	0	0	0	2 (2.0)	2 (1.5)
Lung	0	0	0	0	0	0	0	0	0	0	1 (1.0)	0
Heart	0	0	0	0	0	2 (1.0)	0	0	0	0	2 (2.5)	1 (1.0)
Nerve, Sciatic	0	0	0	0	0	1 (2.0)	0	0	0	0	1 (3.0)	1 (1.0)
Lacrimal Gland	0	0	0	0	0	0	0	0	0	0	1 (1.0)	0
Lymph Node, Mandibular[b]	0	0	0	0	0	1 (2.0)	0	0	0	0	0	0
Epididymis	0	0	0	0	0	1 (3.0)	-	-	-	-	-	-
Seminal Vesicle	0	0	0	0	0	1 (1.0)	-	-	-	-	-	-
Ovary	-	-	-	-	-	-	0	0	0	0	2 (2.0)	0
Uterus	-	-	-	-	-	-	0	0	0	0	2 (3.0)	2 (2.0)
Cervix	-	-	-	-	-	-	0	0	0	0	1 (4.0)	0
Vagina[c]	-	-	-	-	-	-	0	0	0	0	1 (3.0)	0
Subcutaneous Site C	0	0	0	0	0	0	0	0	0	0	1 (1.0)	1 (1.0)
Subcutaneous Site D	0	0	0	0	0	0	0	0	0	0	0	1 (1.0)

a Incidence and mean severity ( ) based on number of animals with finding.
b No. animals examined for mandibular lymph node is four in Group 1 males and females and three Group 5 males.
c No. animals examined for vagina is four in Group 1 females.

Increased incidence and severity of perivascular cellular infiltrates was observed in subcutaneous injection sites at 1 mg/kg/dose (group 4) and higher (see Sponsor's table below). These infiltrates were characterized by cuffing of subcutaneous veins by a mixed cellular infiltrate consisting of lymphocytes, macrophages, and plasma cells, with lower numbers of eosinophils and neutrophils. Infiltrating macrophages occasionally

had vacuolated cytoplasm, considered consistent with intracytoplasmic accumulation of PEG following phagocytosis of the test article. This change was considered test article-related.

Table 48: Cellular Infiltrate in Injection Sites in a 39-Week SC Monkey Toxicity Study (Dosing Phase Final Sacrifice[a])

Sex	Male						Female					
Group	1	2	3	4	5	6	1	2	3	4	5	6
No. Examined	5	3	3	3	4	5	5	3	3	3	4	4
Subcutaneous Site A	2 (1.0)	1 (1.0)	3 (1.0)	3 (1.3)	4 (1.8)	5 (1.4)	1 (1.0)	1 (1.0)	3 (1.0)	3 (2.0)	4 (1.8)	4 (2.3)
Subcutaneous Site B	2 (1.0)	1 (1.0)	3 (1.0)	3 (1.7)	4 (2.0)	5 (2.2)	1 (1.0)	2 (1.0)	3 (1.0)	3 (2.0)	4 (2.3)	4 (2.3)
Subcutaneous Site C	1 (1.0)	2 (1.0)	3 (1.3)	3 (1.3)	3 (2.3)	5 (2.0)	2 (1.0)	3 (1.3)	3 (1.0)	3 (1.3)	4 (1.8)	4 (2.5)
Subcutaneous Site D	1 (1.0)	2 (1.0)	3 (1.0)	2 (1.0)	3 (1.7)	3 (1.0)	0	1 (1.0)	2 (1.0)	2 (1.0)	2 (2.0)	4 (1.3)

a Incidence and mean severity ( ) based on number of animals with finding.

Minimal glomerulopathy and thymic atrophy (minimal to severe) was observed in almost all treatment groups (see table below). However, these changes were not considered to be treatment-related since there was no dose-dependency and similar changes were also observed in the control animals.

As shown in the table below (taken from the study report), there was a dose-dependent increase in incidence and severity in lymphoid nodules in the bone marrow of the sternum (increased lymphopoiesis). This change appeared to be treatment-related in the 7/5/3 mg/kg/dose group (#6), but it may also be related to the arterial inflammation described above, and is therefore considered as possible evidence of an enhanced immune response.

Table 49: Microscopic Findings in Kidney, Bone Marrow, and Thymus in a 39-Week SC Monkey Toxicity Study (Dosing Phase Final Sacrifice[a])

Sex	Male						Female					
Group	1	2	3	4	5	6	1	2	3	4	5	6
No. Examined	5	3[b]	3	3	4[b]	5	5	3	3	3	4	4
Kidney												
Glomerulopathy	0	1 (1.0)	1 (1.0)	1 (1.0)	1 (1.0)	1 (1.0)	1 (1.0)	0	1 (1.0)	1 (1.0)	2 (1.0)	1 (1.0)
Marrow, Sternum												
Nodules, Lymphoid	0	0	1 (1.0)	0	0	3 (1.7)	1 (1.0)	0	0	1 (2.0)	1 (1.0)	3 (1.3)
Thymus												
Atrophy	2 (2.5)	0	2 (3.5)	1 (3.0)	3 (2.3)	3 (3.7)	1 (3.0)	0	0	0	2 (2.5)	2 (2.0)

a Incidence and mean severity ( ) based on number of animals with finding.
b No. examined for thymus in Group 2 and Group 5 males is two and three, respectively.

At the end of the recovery period, increased lymphoid nodules in the bone marrow of the sternum were not completely reversible. The incidence and severity are summarized in the table below (taken from the study report).

Table 50: Microscopic Findings in Bone Marrow and Thymus in a 39-Week SC Monkey Toxicity Study (Recovery Phase Sacrifice[a])

Sex	Male						Female					
Group	1	2	3	4	5	6	1	2	3	4	5	6
No. Examined	3	0	0	0	3	3	3	0	0	0	3	3
Marrow, Sternum												
Nodules, Lymphoid	0	-	-	-	1 (2.0)	1 (1.0)	0	-	-	-	2 (1.0)	0
Thymus												
Atrophy	1 (2.0)	-	-	-	1 (4.0)	2 (2.0)	0	-	-	-	2 (3.0)	1 (1.0)

a Incidence and mean severity ( ) based on number of animals with finding.

Toxicokinetics

Blood samples were collected from non-fasted animals prior to study initiation, during treatment (pre-dose, and 9, 24, 48, and 72 hr post-dose on a weekly basis), and during the recovery period (twice weekly) for analysis of plasma rAvPAL-PEG levels using a sandwich ELISA. The lower limit of quantification (LLOQ) for this assay was 30 ng/ml.

During week 1 of the dosing phase, systemic exposure to rAvPAL-PEG ($AUC_{0\text{-}72hr}$ and C_{max}) was dose proportional. Plasma concentrations in animals treated with 0.01 to 1.0 mg/kg/dose declined to levels that were very close to the LLOQ or <LLOQ following week 2 of the dosing phase. The decline in plasma levels may have been due to the formation of antibodies against rAvPAL-PEG, resulting in accelerated clearance of the drug from plasma. $AUC_{0\text{-}72hr}$ and C_{max} increased with doses up to 3 mg/kg/dose. With the exception of two animals, there was no apparent accumulation when comparing the

$AUC_{0\text{-}72hr}$ from week 39 to week 1. The TK data is summarized in the tables below (taken from the study report).

Table 51: Mean Toxicokinetic Parameters for rAvPAL-PEG in Plasma in a 39-Week SC Monkey Toxicity Study (Week 1)

First week

Dose Group	Dose Level mg/kg	Sex		$AUC_{0\text{-}72\ hr}$ ng-hr/mL	C_{max} ng/mL	Tmax hr
2	0.01	Male	Mean	3054	60	40.0
			SD	1826	24	27.7
			N	3	3	3
		Female	Mean	2497	42	48.0
			SD	2168	36	0.0
			N	3	3	2
		Combined	Mean	2776	51	43.2
			SD	1818	29	20.1
			N	6	6	5
3	0.1	Male	Mean	68219	1335	48.0
			SD	43388	825	24.0
			N	3	3	3
		Female	Mean	21888	481	72.0
			SD	19500	468	--
			N	3	3	2
		Combined	Mean	45053	908	57.6
			SD	39358	761	21.5
			N	6	6	5
4	1.0	Male	Mean	1076245	30538	72.0
			SD	273240	13249	0.0
			N	3	3	3
		Female	Mean	779795	16315	72.0
			SD	27166	2157	0.0
			N	3	3	3
		Combined	Mean	928020	23427	72.0
			SD	237747	11523	0.0

Dose Group	Dose Level mg/kg	Sex		$AUC_{0\text{-}72\ hr}$ ng-hr/mL	C_{max} ng/mL	Tmax hr
			N	6	6	6
5	3	Male	Mean	2618490	53861	48.0
			SD	1223670	32090	19.6
			N	7	7	7
		Female	Mean	2049863	41446	51.4
			SD	368465	7430	9.1
			N	7	7	7
		Combined	Mean	2334176	47654	49.7
			SD	916955	23286	14.8
			N	14	14	14
6	7.0/5.0/3.0[a]	Male	Mean	6399628	139253	54.0
			SD	2228218	46054	11.1
			N	8	8	8
		Female	Mean	5753322	151725	52.0
			SD	1149781	37678	18.1
			N	6	6	6
		Combined	Mean	6122640	144598	53.1
			SD	1814405	41582	13.9
			N	14	14	14

a – Dose for this group was reduced due to toxicity from 7.0 to 5.0 to 3.0 mg/kg

Table 52: Mean Toxicokinetic Parameters for rAvPAL-PEG in Monkey Plasma in a 39-Week SC Toxicity Study (Week 39)

Last Week (39 weeks)

Dose Group	Dose Level mg/kg	Sex		$AUC_{0\text{-}72\ hr}$ ng-hr/mL	C_{max} ng/mL	Tmax hr
2	0.01	Male	Mean	0	0	--
			SD	0	0	--
			N	3	3	--
		Female	Mean	0	0	--
			SD	0	0	--
			N	3	3	--
		Combined	Mean	0.0	0.0	--
			SD	0.0	0.0	--
			N	6	6	0
3	0.1	Male	Mean	1609	115	9.0
			SD	2787	200	--
			N	3	3	1
		Female	Mean	384	47	14.0
			SD	283	19	8.7
			N	3	3	3
		Combined	Mean	996	81	12.8
			SD	1895	132	7.5
			N	6	6	4
4	1.0	Male	Mean	33739	729	9.0
			SD	27179	537	0.0
			N	3	3	3
		Female	Mean	23122	545	27.0
			SD	25337	503	19.7
			N	3	3	3
		Combined	Mean	28431	637	18.0
			SD	24209	476	15.9
			N	6	6	6

Dose Group	Dose Level mg/kg	Sex		$AUC_{0\text{-}72\ hr}$ ng-hr/mL	C_{max} ng/mL	Tmax hr
5	3	Male	Mean	124917	3337	19.7
			SD	209396	4613	7.3
			N	7	7	7
		Female	Mean	2285423	40325	17.6
			SD	3858063	68427	16.0
			N	7	7	7
		Combined	Mean	1205170	21831	18.6
			SD	2854258	50391	12.0
			N	14	14	14
6	7.0/5.0/3.0[a]	Male	Mean	116467	2828	16.5
			SD	189490	4333	8.0
			N	8	8	8
		Female	Mean	190483	4950	33.6
			SD	164691	4572	13.1
			N	5	5	5
		Combined		144935	3644	23.1
				177175	4367	13.0
				13	13	13

a – Dose for this group was reduced due to toxicity from 7.0 to 5.0 to 3.0 mg/kg

Special Evaluation

1. Electron Microscopic Examination

Epoxide-embedded samples of renal cortex and medulla collected from animals in the control and 7/5/3 mg/kg/dose groups at the end of the treatment period and in the control, 3, and 7/5/3 mg/kg/dose groups at the end of the recovery period were prepared for transmission electron microscopy (TEM). However, only renal cortex was examined to characterize morphologic effects in glomeruli and proximal convoluted tubule epithelium. There were no treatment-related ultrastructural changes in the renal cortex (primarily glomerulus and proximal convoluted tubule epithelium).

2. Antibody analysis

Blood samples were collected from non-fasted animals (scheduled and unscheduled sacrifices) before study initiation, during the treatment (weekly or bi-weekly) and recovery periods (every 4 weeks).

All animals treated with rAvPAL-PEG developed measurable anti-rAvPAL-PEG IgG titers between days 15 and 22 of the dosing phase, with a dose-dependent increase in titers throughout the dosing phase. All control animals and animals in the 0.01 mg/kg/dose group were negative for neutralizing antibodies. All other animals, with the exception of one female in the 0.1 mg/kg/dose group and one female in the 7/5/3 mg/kg/dose group (premature sacrifice), were positive for neutralizing antibodies. The correlation between the anti-rAvPAL-PEG IgG antibodies and neutralizing antibodies to rAvPAL-PEG was uncertain. The incidence of animals with anti-rAvPAL-PEG antibodies (IgG and IgM) and neutralizing antibodies are summarized in the table below (taken from the study report).

Table 53: Incidence of Anti-rAvPAL-PEG Positive Animals

		Incidence of Anti-rAvPAL-PEG Positive Animals			
		IgG		IgM	NAb
Group	Dose mg/kg	No. Positive at baseline	No. Positive during study	No. Positive	No. Positive
1	0 (Control)	4/8M, 3/8F	4/8M, 5/8F	0/8M, 4/8F	0/8M, 0/8F
2	0.01	0/3M, 1/3F	3/3M, 3/3F	0/3M, 1/3F	0/3M, 0/3F
3	0.1	1/3M, 0/3F	3/3M, 3/3F	1/3M, 1/3F	3/3M, 2/3F
4	1.0	3/3M, 1/3F	3/3M, 3/3F	2/3M, 2/3F	3/3M, 3/3F
5	3.0	2/7M, 2/7F	7/7M, 7/7F	5/7M, 3/7F	7/7M, 7/7F
6	7.0/5.0/3.0	1/8M, 1/8F	8/8M, 8/8F	7/8M, 6/8F	8/8M, 7/8F

3. Phenylalanine analysis

Blood samples were collected from non-fasted animals pre-treatment (1 and 2-weeks), during the treatment (pre-dose, and 9, 24, 48, 72 hr post-dose on a weekly basis) and recovery periods (twice weekly). Phenylalanine in blood plasma was analyzed using LC-API/MS/MS detection. Phenylalanine-ring-$^{13}C_6$ was used as the internal standard. The Lower Limit of Quantitation (LLOQ) was established at 1 µM.

Following the first dose, plasma phenylalanine (Phe) concentration for animals given ≥1.0 mg/kg/dose was reduced by 51-94% within 9 hours of rAvPAL-PEG administration. Phe levels remained markedly decreased for most animals through day 11 or 15 of the dosing phase. At 0.1 mg/kg/dose, phenylalanine concentration appeared slightly decreased in a few animals during the first week of the dosing phase. Phenylalanine concentration for animals treated with 3 or 7 mg/kg/dose was at or below the LLOQ through days 2 to 11 of the dosing phase. Phenylalanine concentrations returned to control levels by day 22 of the dosing phase and remained at these levels until near the end of the dosing phase for most animals given ≥ 1 mg/kg/dose.

One female in the 3 mg/kg/dose group had consistently low phenylalanine (except at one time-point) throughout the dosing phase, and two females given ≥ 3 mg/kg/dose had notably low phenylalanine concentrations at multiple intervals during the dosing phase. Following dosing on day 267, one male in the 1 mg/kg/dose group and several animals given ≥ 3 mg/kg/dose had markedly decreased phenylalanine concentrations by 9 hours post-dose. Phenylalanine concentrations remained markedly decreased through day 270 of the dosing phase for one male given 3 mg/kg/dose and several females given ≥ 3 mg/kg/dose. Decreased plasma phenylalanine levels were completely reversible during the recovery period.

4. Immunohistochemistry

A. Distribution of rAvPAL-PEG and PEG

Immunohistochemical localization of rAvPAL-PEG and PEG was evaluated in liver (group 6: 7/5/3 mg/kg/dose group only), selected kidney, and/or heart from group 1 (0 mg/kg/dose), group 5 (3 mg/kg/dose) and group 6 males and females as shown in the table below (taken from the study report). rAvPAL-PEG distribution was also evaluated in study monkey livers used as ancillary control tissues.

Covance Study No. 7061-124 Tissue/Blocks to be Sectioned and Stained (16 sections/tissue)

Group (Treatment)	Final Phase Sacrifice Animal Numbers	Sex	Tissues
1 (0 mg/kg/dose)	I04846	F	Heart (block 25) Kidney (block 08)
	I04847	F	Heart (block 26) Kidney (block 09)
	I04815	M	Heart (block 26) Kidney (block 08)
	I04818	M	Heart (block 26) Kidney (block 09)
5 (3.0 mg/kg/dose rAvPAL-PEG)	I04863	F	Heart (block 25) Kidney (block 08)
	I04864	F	Heart (block 25) Kidney (block 08)
	I04831	M	Heart (block 26)
	I04832	M	Kidney (block 08W)
	I04833	M	Kidney (block 08)
	I04834	M	Heart (block 26)
6 (7.0/5.0/3.0 mg/kg/dose rAvPAL-PEG)	I04870	F	Heart (block 26) Kidney (block 09)
	I04872	F	Heart (block 26) Kidney (block 08)
	I04838	M	Heart (block 26)
	I04841	M	Heart (block 26) Kidney (block 09)[a]
	I04842	M	Kidney (block 09)

Tissue sections were stained with rabbit IgG anti-rAvPAL-PEG (also called BP84ex) and rabbit anti-polyethylene glycol (anti-PEG). Test article or PEG was presented in intravascular proteinaceous material, endothelium, interstitial proteinaceous material, and mononuclear cells in group 6 monkey liver and/or groups 5 and 6 monkey heart and kidney. rAvPAL-PEG was also presented in group 6 monkey liver Kupffer cells (and a few hepatocytes). These staining patterns were consistent with biodistribution and transport patterns for macromolecules (including PEGylated moieties) with a limited tissue-specific uptake (e.g., macrophages in multiple tissues, Kupffer cells and to a lesser extent, hepatocytes in liver). rAvPAL-PEG distribution in liver, heart, and kidney is summarized in the tables below (taken from the study report). Vessels with diagnostic changes of arterial inflammation, based on HE staining, were judged to be

"affected vessels" because the histologic changes in the vascular wall. Vessels with no alterations or perivascular/interstitial infiltrates only (i.e., no involvement of the vascular wall) were judged to be "unaffected vessels."

Text-Table 1: rAvPAL-PEG Biodistribution in Monkey Liver

IHC Comparative Increase Scores: <1↑ very limited increase; 1↑ = minimal increase; 2↑ = slight increase; 3↑ = moderate increase; 4↑ = marked (intense) increase; - = Negative = no staining or no increase; NE = not evaluated (or tissue element not present for evaluation).

Group (Dose Level mg/kg/dose)	Animal # & Sex	Intravascular Proteinic Material		Endothelium		Interstitial Proteinic Material		Kupffer Cells		Hepatocytes		Mononuclear Cells	
		Anti-rAvPAL-PEG	Anti-PEG	Anti-rAvPAL-PEG	Anti-PEG	Anti-rAvPAL-PEG	Anti-PEG	Anti-rAvPAL-PEG	Anti-PEG	Anti-rAvPAL-PEG	Anti-PEG	Anti-rAvPAL-PEG	Anti-PEG
Group 1 (Control)	I04814 (M)	-	-	-	-	-	-	-	-	-	-	-	-
	I04846 (F)	-	-	-	-	-	-	-	-	-	-	-	-
Group 6 (7.0/5.0/3.0 mg/kg/dose rAvPAL-PEG)	I04841 (M)	<1↑	1-2↑	1-2↑	2↑	-	<1↑	1↑	2↑	<1↑	1-2↑	<1↑	1↑
	I04872 (M)	-	2↑	1↑	2↑	-	1↑	<1↑	2↑	-	1↑	<1↑	1↑

Text-Table 2: rAvPAL-PEG Biodistribution in Monkey Heart

IHC Comparative Increase Scores: <1↑ (very) minimal, limited or trace increase; 1↑ = minimal increase; 2↑ = mild increase; 3↑ = moderate increase; 4↑ = marked (intense) increase; - = Negative = no staining or no increase; NE = not evaluated (or tissue element not present for evaluation). Abbreviations: (I) = intima; (M) = media; (I>M) = more staining in intima than media; (I/M) = equivalent intima and media staining.

Group (Dose Level mg/kg/dose)	Animal # & Sex	Intravascular Material		Endothelium*		Interstitial Material		Intima (I)/Media (M), Muscular Artery/Arteriole, Unaffected Vessels		Intima (I)/Media (M), Muscular Artery/Arteriole, Affected Vessels		Great Vessel Outflow Tract(s)		Mononuclear Cells	
		Anti-rAvPAL-PEG	Anti-PEG	Anti-rAvPAL-PEG	Anti-PEG	Anti-rAvPAL-PEG	Anti-PEG	Anti-rAvPAL-PEG	Anti-PEG	Anti-rAvPAL-PEG	Anti-PEG	Anti-rAvPAL-PEG	Anti-PEG	Anti-rAvPAL-PEG	Anti-PEG
Group 1 (Control)	I04815 (M)	-	-	-	-	-	-	-	-	NE	NE	-	-	-	-
	I04818 (M)	-	-	-	-	-	-	-	-	NE	NE	-	-	-	-
	I04846 (F)	-	-	-	-	-	-	-	-	NE	NE	-	-	-	-
	I04847 (F)	-	-	-	-	-	-	-	-	NE	NE	-	-	-	-
Group 5 (3.0 mg/kg/dose rAvPAL-PEG)	I04831 (M)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	I04834 (M)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	I04863 (F)	<1↑	1-2↑	<1↑	1↑	-	1↑	-	<1↑ (I)	<1↑ (I>M)	1-2↑ (I/M)	<1↑	1↑	<1↑	2↑
	I04864 (F)	-	2↑	<1↑	1↑	-	<1↑	<1↑ (I>M)	1↑ (I>M)	<1↑ (I/M)	2↑ (I/M)	<1↑	1-2↑	-	1↑
Group 6 (7.0/5.0/3.0 mg/kg/dose rAvPAL-PEG)	I04838 (M)	-	1↑	-	<1↑	-	<1↑	-	1↑ (I>M)	NE	NE	-	<1↑	-	-
	I04841 (M)	-	-	-	<1↑	-	-	-	<1↑ (I)	-	<1↑ (I)	-	<1↑ (I,M)	-	<1↑

Text-Table 2: rAvPAL-PEG Biodistribution in Monkey Heart

IHC Comparative Increase Scores: <1↑ (very) minimal, limited or trace increase; 1↑ = minimal increase; 2↑ = mild increase; 3↑ = moderate increase; 4↑ = marked (intense) increase; - = Negative = no staining or no increase; NE = not evaluated (or tissue element not present for evaluation). Abbreviations: (I) = intima; (M) = media; (I>M) = more staining in intima than media; (I/M) = equivalent intima and media staining.

Group (Dose Level mg/kg/dose)	Animal # & Sex	Intravascular Material		Endothelium*		Interstitial Material		Intima (I)/Media (M), Muscular Artery/Arteriole, Unaffected Vessels		Intima (I)/Media (M), Muscular Artery/Arteriole, Affected Vessels		Great Vessel Outflow Tract(s)		Mononuclear Cells	
		Anti-rAvPAL-PEG	Anti-PEG	Anti-rAvPAL-PEG	Anti-PEG	Anti-rAvPAL-PEG	Anti-PEG	Anti-rAvPAL-PEG	Anti-PEG	Anti-rAvPAL-PEG	Anti-PEG	Anti-rAvPAL-PEG	Anti-PEG	Anti-rAvPAL-PEG	Anti-PEG
	I04870 (F)	<1↑	<1↑	-	-	-	<1↑	-	<1↑ (I)	<1↑ (I>M)	1-2↑ (I,M)	<1↑	1-2↑	-	<1↑
	I04872 (F)	-	<1↑	-	-	-	<1↑	-	-	NE	NE	-	-	-	-

*Alterations evident in HE slide(s) examined (b) (4) 7061-124 Study Pathologist but not observed in adjunctive HE slides examined at (b) (4) (likely section:section variation).

Text-Table 3: rAvPAL-PEG Biodistribution in Monkey Kidney

IHC Comparative Increase Scores: <1↑ (very) minimal, limited or trace increase; 1↑ = minimal increase; 2↑ = mild increase; 3↑ = moderate increase; 4↑ = marked (intense) increase; - = Negative = no staining or no increase; NE = not evaluated (or tissue element not present for evaluation). Abbreviations: (I) = intima; (M) = media; (I>M) = more staining in intima than media; (I/M) = equivalent intima and media staining.

Group (Dose Level mg/kg/dose)	Animal # & Sex	Intravascular Material		Endothelium		Interstitial Material		Mesangium		Intima (I)/Media (M), Muscular Artery/Arteriole, Unaffected Vessels		Intima (I)/Media (M), Muscular Artery/Arteriole, Affected Vessels		Mononuclear Cells	
		Anti-rAvPAL-PEG	Anti-PEG	Anti-rAvPAL-PEG	Anti-PEG	Anti-rAvPAL-PEG	Anti-PEG	Anti-rAvPAL-PEG	Anti-PEG	Anti-rAvPAL-PEG	Anti-PEG	Anti-rAvPAL-PEG	Anti-PEG	Anti-rAvPAL-PEG	Anti-PEG
Group 1 (Control)	I04815 (M)	-	-	-	-	-	-	-	-	-	-	NE	NE	-	-
	I04818 (M)	-	-	-	-	-	-	-	-	-	-	NE	NE	-	-
	I04846 (F)	-	-	-	-	-	-	-	-	-	-	NE	NE	-	-
	I04847 (F)	-	-	-	-	-	-	-	-	-	-	NE	NE	-	-
Group 5 (3.0 mg/kg/dose rAvPAL-PEG)	I04832 (M)	-	1↑	-	1↑	-	<1↑	-	1-2↑	-	<1↑ (I)	<1↑ (I)	<1↑ (I)	-	-
	I04833 (M)	-	1↑	-	-	-	-	-	-	-	-	-	-	-	-
	I04863 (F)	<1↑	1-2↑	-	-	1↑	1↑	<1↑	1-2↑	-	1↑ (I>M)	<1↑ (I)	2↑ (I/M)	<1↑	1↑
	I04864 (F)	<1↑	<2-3↑	-	1↑	-	1-2↑	<1↑	2↑	-	1↑ (I)	NE	NE	-	-
Group 6 (7.0/5.0/3.0 mg/kg/dose rAvPAL-PEG)	I04841 (M)	<1↑	1-2↑	-	1↑	-	<1↑	<1↑	1↑	-	<1↑ (I>M)	<1↑ (I/M)	1-2↑ (I/M)	-	-
	I04842 (M)	<1↑	1↑	-	-	-	-	<1↑	1-2↑	-	<1↑ (I/M)	-	<1↑ (I/M)	-	-
	I04870 (F)	<1↑	1-2↑	-	1↑	-	1-2↑	<1↑	1↑	-	1↑ (I)	<1↑ (I/M)	1-2↑ (I/M)	-	<1↑
	I04872 (F)	<1↑	1↑	-	-	-	-	<1↑	1↑	-	<1↑ (I)	-	1↑ (I)	-	-

B. Distribution of immune complex components

The potential immune complex components (endogenous monkey immunoglobulins IgG, IgM, and complement components SC5b-9 (membrane-attack complex), and C3) was evaluated in routine paraffin-embedded kidney and heart tissues (granular deposits in affected and unaffected vessels) selected from animals in group 1 (control), group 5 (3 mg/kg/dose) and group 6 (7/5/3 mg/kg/dose). The table below (taken from the study report) summarizes the results of monkey IgG, IgM, C3, and SC5b-9 staining in affected and unaffected vessels (arteries/arterioles) in monkey kidney.

There was no staining with anti-rAvPAL-PEG or anti-PEG in control kidneys. However, there were minor increases in IgG, IgM, or C3 granular deposits in unaffected vessels (usually pelvic arteries) in treated and untreated monkeys, usually in association with very low-grade (subdiagnostic) atheromatous changes (increased intimal basophilia, vacuolation or protrusion). There were no affected vessels for evaluation in group 1 monkey kidneys.

The heart (2/2 females in group 5, 1/2 males in group 6, 1/2 females in group 6) and kidney (1/2 females in group 5, 1/2 males in group 6, 2/2 females in group 6) had increased granular deposit staining with anti-PEG, IgG, and/or IgM reagents in affected vessels, where immune complex components were presented in the vascular wall (intima and/or media) of muscular arteries/arterioles, as compared to unaffected vessels with no alterations or perivascular/interstitial infiltrates. Increases in anti-rAvPAL-PEG and/or anti-PEG staining in intima and/or media in coronary or renal arteries or arterioles were associated with arterial inflammation. Granular deposits were less often stained with anti-rAvPAL-PEG, indicating either that this stain was less sensitive in heart and kidney or that the stained material was primarily the PEG component.

Similar immunohistologic changes were observed in group 6 kidney vessels and/or liver/gall bladder vessels in the feasibility portion of this study.

Text-Table 5: Comparison of rAvPAL-PEG, IgG, IgM, C3 and SC5b-9 Staining Patterns in Arteries/Arterioles in Monkey Kidney

Histologic Scores: (b) (4) assigned histologic scores of 1 = minimal, 2 = slight, 3 = moderate, 4 = moderately severe/marked, 5 = severe. **IHC Comparative Increase Scores:** <1↑ (very) minimal, limited or trace increase; 1↑ = minimal increase; 2↑ = mild increase; 3↑ = moderate increase; 4↑ = marked (intense) increase; - = Negative = no staining or no increase; NE = not evaluated (or tissue element not present for evaluation).
Abbreviations: Inflam = inflammation; (I) = intima; (M) = media; (I>M) = more staining in intima than media; (I/M) = equivalent intima and media staining.

Group (Dose Level mg/kg/dose)	Animal # & Sex	Histologic Findings, Vascular Wall, Affected Vessels	Granular Deposits, Intima (I)/Media (M), Muscular Artery/Arteriole, Unaffected Vessels						Granular Deposits, Intima (I)/Media (M), Muscular Artery/Arteriole, Affected Vessels					
			Anti-rAvPAL-PEG	Anti-PEG	IgG	IgM	C3	SC5b-9	Anti-rAvPAL-PEG	Anti-PEG	IgG	IgM	C3	SC5b-9
Group 1 (Control)	I04815 (M)	None	-	-	<1↑ (I)	1↑ (I/M)	-	-	NE	NE	NE	NE	NE	NE
	I04818 (M)	None	-	-	-	<1↑ (I>M)	<1↑ (I)	-	NE	NE	NE	NE	NE	NE
	I04846 (F)	None	-	-	<1↑ (I>M)	1↑ (I>M)	<1↑ (I>M)	-	NE	NE	NE	NE	NE	NE
	I04847 (F)	None	-	-	<1↑ (I)	1↑ (I>M)	<1↑ (I>M)	-	NE	NE	NE	NE	NE	NE
Group 5 (3.0 mg/kg/dose rAvPAL-PEG)	I04832 (M)	Inflam, arterial 1	-	<1↑ (I)	<1↑ (I)	1↑ (I/M)	-	-	<1↑ (I)	<1↑ (I)	-	1↑ (I/M)	<1↑ (I)	-
	I04833 (M)	None	-	-	<1↑ (I)	1↑ (I)	<1↑ (I)	-	NE	NE	NE	NE	NE	NE
	I04863 (F)	Inflam, arterial 4	-	1↑ (I>M)	1↑ (I)	1-2↑ (I/M)	1↑ (I)	-	<1↑ (I>M)	2↑ (I/M)	2↑ (I/M)	2-3↑ (I/M)	1↑ (I/M)	<1↑ (M)
	I04864 (F)	Inflam, arterial 1	-	1↑ (I)	-	<1↑ (I)	-	-	NE*	NE*	NE*	NE*	-	-
Group 6 (7.0/5.0/3.0 mg/kg/dose rAvPAL-PEG)	I04841 (M)	Inflam, arterial 1	-	<1↑ (I>M)	-	1↑ (I)	-	-	<1↑ (I/M)	1-2↑ (I/M)	1↑ (I)	2↑ (I/M)	<1↑ (I)	-
	I04842 (M)	None	-	<1↑ (I/M)	<1↑ (I)	1↑ (I/M)	<1↑ (I)	-	-	<1↑ (I/M)	-	1↑ (I/M)	<1↑ (I)	-

Text-Table 5: Comparison of rAvPAL-PEG, IgG, IgM, C3 and SC5b-9 Staining Patterns in Arteries/Arterioles in Monkey Kidney

Histologic Scores: (b) (4) assigned histologic scores of 1 = minimal, 2 = slight, 3 = moderate, 4 = moderately severe/marked, 5 = severe. **IHC Comparative Increase Scores:** <1↑ (very) minimal, limited or trace increase; 1↑ = minimal increase; 2↑ = mild increase; 3↑ = moderate increase; 4↑ = marked (intense) increase; - = Negative = no staining or no increase; NE = not evaluated (or tissue element not present for evaluation). **Abbreviations:** Inflam = inflammation; (I) = intima; (M) = media; (I>M) = more staining in intima than media; (I/M) = equivalent intima and media staining.

Group (Dose Level mg/kg/dose)	Animal # & Sex	Histologic Findings, Vascular Wall, Affected Vessels	Granular Deposits, Intima (I)/Media (M), Muscular Artery/Arteriole, Unaffected Vessels						Granular Deposits, Intima (I)/Media (M), Muscular Artery/Arteriole, Affected Vessels					
			Anti-rAvPAL-PEG	Anti-PEG	IgG	IgM	C3	SC5b-9	Anti-rAvPAL-PEG	Anti-PEG	IgG	IgM	C3	SC5b-9
	I04870 (F)	Inflam, arterial 3	-	1↑ (I)	1↑ (I/M)	1↑ (I/M)	<1↑ (I)	-	<1↑ (I/M)	1-2↑ (I/M)	1-2↑ (I/M)	2↑ (I/M)	1↑ (I)	-
	I04872 (F)	Inflam, arterial 2**	-	<1↑ (I)	<1↑ (I)	<1↑ (I)	<1↑ (I)	-	-	1↑ (I)	1↑ (I/M)	2↑ (I/M)	1↑ (I)	-

*Affected vessel(s) were only present in the C3 and SC5b-9 slides for Group 5 male I04864. **Pelvic vein inflammation also present in Group 6 female I04872.

Thus, the Sponsor concluded that the histologic and immunohistochemical alterations in liver, kidney, and heart in group 5 (3 mg/kg/dose) and group 6 (7/5/3 mg/kg/dose) were consistent with, but not diagnostic for, immune complex-mediated arterial inflammation based on the following observations:

• Arterial inflammation observed primarily at branch points in arteries (and arterioles)
• Hypertrophy and proliferation of intima (endothelium) and media
• Macrophage and lymphocyte infiltrates into arterial/arteriolar walls (mural infiltrates, intima and/or media)
• Increased IgM deposition or retention (intima and/or media) associated with intimal (and/or medial) proliferation and inflammation
• Presence of PEG and/or rAvPAL-PEG in arterial/arteriolar walls (intima and/or media)
• Morphologic characteristics of immunohistochemical staining (granular deposits).

Dosing Solution Analysis

All dose formulations were stored at room temperature until dosing, within 3 hours of preparation. All dose solutions were stable from time 0 to 3 hr post-preparation, and were homogeneous. The concentrations of dose solutions were all within 10% of the nominal concentrations, with the exception of the week 13 sample for Group 4 (1 mg/ml) which was 16% lower than nominal concentration, and week 26 samples for Groups 3 (0.1 mg/ml) and 4 (3 mg/ml) which measured 20% and 19% lower than nominal concentrations, respectively.

7 Genetic Toxicology

N/A

8 Carcinogenicity

The Sponsor submitted an assessment of carcinogenicity potential, but did not conduct any carcinogenicity studies.

Pegvaliase is a homotetramer of recombinant phenylalanine ammonia lyase (rAv-PAL) conjugated with polyethylene glycol (PEG, MW = 20 kDa). Phenylalanine ammonia

lyase converts phenylalanine to ammonia and trans-cinnamic acid. Ammonia is metabolized to urea in the liver, and trans-cinnamic acid is excreted in urine. The mechanism of action does not raise a concern regarding the potential for neoplasm induction or tumor promotion. In addition, a literature search did not reveal evidence of carcinogenicity for PAL.

In rats and mice, oral administration of PEG with varying molecular weights inhibited colon carcinogenesis induced by chemicals (e.g. azoxymethane, N-methyl-N-nitrosourea, 2-amino-3,4-dimethylimidazo(4,5-f)quinolone) (Corpet DE, et al. Cancer Research 2000, 60: 3160-3164; Karlsson PC, et al. Cancer Letters 2005, 223: 203-209).

Furthermore, there were no proliferative lesions in the 26-week rat and 39-week monkey studies, in which the animals were treated with rAvPAL-PEG at doses up to 25 mg/kg twice weekly in rats and 3 mg/kg twice weekly in monkeys via SC administration. Based on a weight-of-evidence approach, the carcinogenic potential of rAvPAL-PEG appears to be low.

CAC Concurrence (y/n): Yes
CAC Recommendations: None
Comments: None

9 Reproductive and Developmental Toxicology

9.1 Fertility and Embryo-Fetal Development

Study title: Fertility and Embryo-fetal Development Study of rAvPAL-PEG Administered by Subcutaneous Injection in Rats

Study no.:	BMN165-12-037
Study report location:	N/A
Conducting laboratory and location:	(b) (4)
Date of study initiation:	November 1, 2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	2011-165-15-111511; 100%

Key Study Findings

rAvPAL-PEG was administered once daily via subcutaneous injection to male and female rats at doses of 0, 2, 8 or 20 mg/kg/day starting before cohabitation with continued treatment through mating, implantation and closure of the hard palate. rAvPAL-PEG at 20 mg/kg/day produced significant reductions in maternal body weight, body weight gain, and food consumption. rAvPAL-PEG had no effects on mating and fertility parameters in either sex, total sperm count, sperm motility, sperm density, or

estrous cycling. rAvPAL-PEG at 8 and/or 20 mg/kg/day significantly reduced the number of corpora lutea, number of implantations, litter size (20 mg/kg/day group only) and live fetuses (20 mg/kg/day group only). The reduction in litter size was secondary to the reduced number of corpora lutea. Fetal weights in the 20 mg/kg/day group were also significantly reduced. rAvPAL-PEG at 8 and 20 mg/kg/day produced increases in the number of litters and fetuses with alterations. However, there were no treatment-related malformations. Fetal exposure to rAvPAL-PEG was evident in the 20 mg/kg/day group. The NOAEL for paternal and maternal toxicity was 8 mg/kg/day, based on decreased body weight, body weight gain, and food consumption at 20 mg/kg /day. The NOAEL for male fertility was 20 mg/kg/day, and the NOAEL for female fertility was 8 mg/kg/day based on the decrease in corpora lutea at 20 mg/kg/day. The NOAEL for embryo-fetal development was 8 mg/kg/day, based on the reduced fetal weights at 20 mg/kg/day.

Methods

Doses:	0 (vehicle), 2, 8, and 20 mg/kg
Frequency of dosing:	Once daily
Dose volume:	2.5 ml/kg
Route of administration:	Subcutaneous injection
Formulation/Vehicle:	rAvPAL-PEG was dissolved in 10 mM TRIS, 135 mM sodium chloride, 1 mM trans-cinnamic acid, and 1 mM ammonia at concentrations of 0.8, 3.2 or 8 mg/ml (pH 7.0 ± 0.5).
Species/Strain:	Sprague-Dawley [Crl:CD(SD)]
Number/Sex/Group:	25
Satellite groups:	TK: 3/sex/group
Study design:	See table below
Deviation from study protocol:	Deviations have no impact on results and data interpretation.

Males were dosed beginning at 41 days (approximately 6 weeks) before cohabitation, with continuation of dosing through cohabitation (mating) until the day before euthanasia, for a total of at least 77 doses administered. Females were dosed beginning at 28 days (approximately 4 weeks) before cohabitation, with continuation of dosing through cohabitation and through Day 17 of presumed gestation (GD 17), for a total of at least 47 doses administered. The study design is shown in the table below (taken from the study report):

Text Table 1
Experimental Design

Group No.	Test Material	Dose Level (mg/kg/day)	Concentration (mg/mL)	Dose Volume (mL/kg)	No. of Main Study Rats		No. of Toxicokinetic Rats[a]	
					M	F	M	F
1	Control Article	0	0	2.5	25	25	3	3
2	rAvPAL-PEG	2	0.8	2.5	25	25	3	3
3	rAvPAL-PEG	8	3.2	2.5	25	25	3	3
4	rAvPAL-PEG	20	8	2.5	25	25	3	3

[a] Toxicokinetic rats were primarily used for AvPAL-PEG exposure and phenylalanine biomarker evaluations in plasma.

Observations and Results

Mortality

There were no treatment-related deaths. One control male and one female in the 2 mg/kg/day (low-dose) group were euthanized on study day 5 (SD 5) or 26 due to adverse clinical observations resulting from an injury. Necropsy revealed a dark red gelatinous material in the thoracic and right axillary regions, and enlarged spleen in the male, and enlarged spleen, enlarged thymus, and a cloudy red fluid in the thoracic cavity in the female. The findings in the female were presumed due to an injury during dosing.

Two females in the 20 mg/kg/day group (high-dose) were delivered on GD 21 and euthanized. There were no treatment-related changes in clinical signs, body weight, food consumption, or necropsy findings. Three of 15 pups delivered by female # 2700 had a large nasal-frontal suture and incompletely ossified squamosal bones, and one additional pup had incompletely ossified squamosal bones.

Clinical Signs

There were no clear treatment-related clinical signs for either sex.

Body Weight

rAvPAL-PEG at 20 mg/kg/day produced significant reductions in body weight, body weight gain, and food consumption. These changes were treatment-related.

Males:

Male rats in the high-dose group had lower body weight over the treatment period and the reduced body weight reached statistical significance during dosing days 41 to 78 (↓up to 8.3% on SD 78; $p \leq 0.01$). The reduction in body weight was duration dependent.

High-dose males also had reduced body weight gain during the treatment period. The significant reduction in body weight gain was observed on SD 41, 57, 64, 71 and 78 (↓up to 48.1% on DS 36-41; $p \leq 0.01$).

Females:

Overall, high-dose females had significantly reduced body weight (↓up to 6.5%; $p \leq 0.01$) and body weight gain (↓up to 63.8%; $p \leq 0.01$) during the pre-cohabitation period.

During the gestation period, high-dose females had significantly reduced body weight (↓up to 14.7%; $p \leq 0.01$) and body weight gain (↓up to 49.8%; $p \leq 0.01$).

Feed Consumption

Males:

Throughout the treatment period, significant reduction in absolute (g/day) and relative (g/kg/day) food consumption was observed in the high-dose males (↓up to 13.4% for absolute food consumption; $p \leq 0.01$; ↓up to 7.4% for relative food consumption; $p \leq 0.01$). Thus, the reduced body weight and body weight gain in the high-dose males was associated with reduced food consumption.

Females:

During the pre-cohabitation period, high-dose females had significantly reduced absolute (g/day) food consumption (↓up to 12.6%; $p \leq 0.01$) and relative food consumption (g/kg/day) (↓up to 7%; $p \leq 0.01$).

During the gestation period, reduced absolute food consumption (↓up to 21.3%; $p \leq 0.01$) was observed in the high-dose females. There was no significant effect on relative food consumption in the high-dose females.

Toxicokinetics

Plasma Concentrations of rAvPAL-PEG (ng/mL) and Phenylalanine (μM)

In general, exposure to rAvPAL-PEG was dose-dependent, and appeared to be higher in females during the treatment period. The plasma level of test-article tended to increase through the treatment period in the high-dose group. Fetal exposure to rAvPAL-PEG was evident in the 20 mg/kg/day group in one of two litters evaluated. The fetal plasma level was 202 ± 286 ng/ml on GD 21 after no treatment for 4 days.

Phenylalanine (Phe) level in the low-dose males (54.6 μM on SD 79) was comparable to the control values (67.2 μM on SD 79) throughout the dosing period. rAvPAL-PEG at 8 and 20 mg/kg/day markedly reduced male plasma Phe levels during the dosing period (67.2 μM, 15.6 μM, and 0 μM (LLOQ: 1 μM) in the control, 8, and 20 mg/kg/day male groups, respectively, on SD 79).

Phe levels were consistently reduced in the low-dose females (29.4 µM on GD 21, as compared to 47.2 µM in the control females) throughout the dosing period (pre-cohabitation and gestation periods). The Phe levels in the mid- and high-dose female groups remained constantly lower after no treatment for 4 days (47.2 µM, 10.8 µM and 0.35 µM on GD 21, in the control, mid- and high-dose groups, respectively).

Necropsy

Fertility Parameters (Mating/Fertility Index, Corpora Lutea, Preimplantation Loss, etc.)

Males:

rAvPAL-PEG had no effects on male mating and fertility parameters (i.e. number of days in cohabitation, number of rats that mated, and fertility index) as shown in the table below (taken from the study report).

TABLE 6 (PAGE 1): MATING AND FERTILITY - SUMMARY - MALE RATS

GROUP		1	2	3	4
TEST MATERIAL		CONTROL ARTICLE	rAvPAL-PEG	rAvPAL-PEG	rAvPAL-PEG
DOSE LEVEL (MG/KG/DAY)		0	2	8	20
RATS IN COHABITATION	N	24a	24b	25	25
DAYS IN COHABITATION c	MEAN±S.D.	2.7 ± 1.0	2.4 ± 1.0	2.7 ± 1.0	2.6 ± 1.7
RATS THAT MATED c	N(%)	24(100.0)	24(100.0)	25(100.0)	25(100.0)
FERTILITY INDEX d,e	N/N	23/24	24/24	23/25	23/25
	(%)	(95.8)	(100.0)	(92.0)	(92.0)
RATS WITH CONFIRMED MATING DATES	N	24	24	25	25
MATED WITH FEMALE					
DAYS 1-7	N(%)	24(100.0)	24(100.0)	25(100.0)	24(96.0)
DAYS 8-14	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.0)
RATS PREGNANT/RATS IN COHABITATION e	N/N	23/24	24/24	23/25	23/25
	(%)	(95.8)	(100.0)	(92.0)	(92.0)

a. Rat 2510 was euthanized on Day 5 of study due to adverse clinical observations.
b. Excludes values for rat 2550, which was not assigned to cohabitation because there were no available female rats.
c. Includes only one mating for each male rat.
d. Number of pregnancies/number of rats that mated.
e. Includes only one pregnancy for each rat that impregnated more than one female rat.

There were no treatment-related effects on total sperm count, sperm motility, or sperm density. The values for number and percent motile sperm, number of non-motile sperm, and total sperm count from the vas deferens and cauda epididymal sperm count and density are presented in the table below (taken from the study report).

TABLE 10 (PAGE 1): SPERM MOTILITY, COUNT, DENSITY AND SPERMATID COUNT - SUMMARY - MALE RATS

GROUP		1	2	3	4
TEST MATERIAL		CONTROL ARTICLE	rAvPAL-PEG	rAvPAL-PEG	rAvPAL-PEG
DOSE LEVEL (MG/KG/DAY)		0	2	8	20
RATS TESTED	N	24a	25	25	25
VAS DEFERENS SPERM MOTILITY					
NUMBER MOTILE	MEAN±S.D.	432.2 ± 146.9	480.0 ± 101.2	443.3 ± 112.7	399.6 ± 118.8
MOTILE PERCENT	MEAN±S.D.	90.5 ± 12.3	91.4 ± 5.5	91.9 ± 4.7	93.4 ± 3.9
STATIC COUNT (NONMOTILE)	MEAN±S.D.	40.6 ± 32.5	46.6 ± 35.1	41.5 ± 31.6	30.8 ± 29.2
TOTAL COUNT b	MEAN±S.D.	472.8 ± 150.7	526.7 ± 117.1	484.8 ± 133.0	430.4 ± 138.4
CAUDA EPIDIDYMAL SPERM COUNT					
SPERM COUNT c	MEAN±S.D.	339.4 ± 95.6	373.8 ± 119.2	353.4 ± 105.4	315.3 ± 87.9
SPERM DENSITY d	MEAN±S.D.	1224.03 ± 293.94	1357.72 ± 399.59	1311.02 ± 344.05	1257.88 ± 329.11

a. Rat 2510 was euthanized on Day 5 of study due to adverse clinical observations.
b. Sum of number motile and static count. Groups of five fields were evaluated until a sperm count of at least 200 was achieved or 20 fields were evaluated.
c. Sperm count used in the calculation of sperm density. Twenty fields were evaluated.
d. The sperm density was calculated by dividing the sperm count by the volume in the image area (76.6 x 10^{-6} mL), multiplying by 2 (dilution factor + 1) and multiplying by 10^{6} to obtain the sperm concentration (million sperm/mL). The calculated sperm concentration value (rounded to 1 decimal place) was multiplied by 50 (volume) and divided by the weight of the left cauda epididymis (see APPENDIX 9 for the weight of the left cauda epididymis) to obtain the sperm density (million sperm/gram of tissue). The calculated value will vary by approximately 0.8% from the Computer Automated Sperm Analysis because the digital image evaluated is slightly smaller (4 pixels) than the actual field causing a slight underestimate of the actual volume and an overestimate of the concentration.

Females:

rAvPAL-PEG at up to 20 mg/kg/day had no effects on estrous cycling or mating and fertility parameters in females, as shown in the table below (taken from the study report).

TABLE 21 (PAGE 2): ESTROUS CYCLING, MATING AND FERTILITY - SUMMARY - FEMALE RATS

GROUP		1	2	3	4
TEST MATERIAL		CONTROL ARTICLE	rAvPAL-PEG	rAvPAL-PEG	rAvPAL-PEG
DOSE LEVEL (MG/KG/DAY)a		0	2	8	20
MATING OBSERVATIONS					
RATS IN COHABITATION	N	25	24	25	25
DAYS IN COHABITATION	MEAN±S.D.	2.6 ± 1.0	2.4 ± 1.0	2.7 ± 1.0	2.6 ± 1.7
RATS THAT MATED	N(%)	25(100.0)	24(100.0)	25(100.0)	25(100.0)
FERTILITY INDEX b	N/N	24/25	24/24	23/25	23/25
	(%)	(96.0)	(100.0)	(92.0)	(92.0)
RATS WITH CONFIRMED MATING DATES	N	25	24	25	25
MATED BY FIRST MALE					
DAYS 1-7	N(%)	25(100.0)	24(100.0)	25(100.0)	24(96.0)
DAYS 8-14	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.0)
RATS PREGNANT/RATS IN COHABITATION	N/N	24/25	24/24	23/25	23/25
	(%)	(96.0)	(100.0)	(92.0)	(92.0)

[ ] = NUMBER OF VALUES AVERAGED
a. Dose administration occurred once daily beginning 28 days before cohabitation, during cohabitation and continued until Day 17 of gestation.
b. Number of pregnancies/number of rats that mated.

Ovarian and Uterine Examinations

rAvPAL-PEG at 8 and/or 20 mg/kg/day significantly reduced the number of corpora lutea (↓7.96% and ↓11.7% in the 8 and 20 mg/kg/day groups, respectively) and implantations (↓9.5% and ↓13.9% in the 8 and 20 mg/kg/day groups, respectively), litter size (↓13.5% in the 20 mg/kg/day group only) and live fetuses (12.8 ± 2.6 in the 20 mg/kg/day group vs 14.8 ± 1.7 in controls). At 20 mg/kg/day, fetal weights (combined, male and female) were also significantly reduced (↓up to 10%; p ≤ 0.01) compared to controls. The data from ovarian and uterine examinations is summarized in the tables below (taken from the study report).

TABLE 23 (PAGE 1): CAESAREAN-SECTIONING OBSERVATIONS - SUMMARY - FEMALE RATS

GROUP		1	2	3	4
TEST MATERIAL		CONTROL ARTICLE	rAvPAL-PEG	rAvPAL-PEG	rAvPAL-PEG
DOSE LEVEL (MG/KG/DAY)a		0	2	8	20
RATS TESTED	N	25	24b	25	25
PREGNANT	N(%)	24(96.0)	24(100.0)	23(92.0)	23(92.0)
DELIVERED AND EUTHANIZED	N(%)	0(0.0)	0(0.0)	0(0.0)	2(8.7)c,d
RATS PREGNANT AND CAESAREAN-SECTIONED ON DAY 21 OF GESTATION	N	24	24	23	21
CORPORA LUTEA	MEAN±S.D.	16.3 ± 1.6	15.7 ± 2.8	15.0 ± 2.2*	14.4 ± 2.2**
IMPLANTATIONS	MEAN±S.D.	15.8 ± 1.5	14.4 ± 2.5	14.3 ± 1.9*	13.6 ± 2.5**
% PREIMPLANTATION LOSS	MEAN±S.D.	3.4 ± 5.3	7.9 ± 10.6	4.1 ± 8.5	5.8 ± 9.2
LITTER SIZES	MEAN±S.D.	14.8 ± 1.7	13.6 ± 2.8	13.3 ± 2.0	12.8 ± 2.6*
LIVE FETUSES	N	355	326	306	269
	MEAN±S.D.	14.8 ± 1.7	13.6 ± 2.8	13.3 ± 2.0	12.8 ± 2.6*
DEAD FETUSES	N	0	0	0	0
RESORPTIONS	MEAN±S.D.	1.0 ± 1.0	0.9 ± 1.5	1.0 ± 1.1	0.8 ± 1.2
EARLY RESORPTIONS	N	23	20	17	15
	MEAN±S.D.	1.0 ± 1.0	0.8 ± 1.5	0.7 ± 0.9	0.7 ± 1.2
LATE RESORPTIONS	N	0	1	5	1
	MEAN±S.D.	0.0 ± 0.0	0.0 ± 0.2	0.2 ± 0.8	0.0 ± 0.2
% POSTIMPLANTATION LOSS	MEAN±S.D.	6.1 ± 6.3	5.8 ± 10.6	6.6 ± 7.7	5.4 ± 8.8

% PREIMPLANTATION LOSS = [(NUMBER OF CORPORA LUTEA - NUMBER OF IMPLANTATIONS) / NUMBER OF CORPORA LUTEA] x 100
% POSTIMPLANTATION LOSS = [(NUMBER OF IMPLANTATIONS - NUMBER OF LIVE FETUSES) / NUMBER OF IMPLANTATIONS] x 100
a. Dose administration occurred once daily beginning 28 days before cohabitation, during cohabitation and continued until Day 17 of gestation.
b. Rat 2643 was euthanized prior to cohabitation on Day 26 of study due to adverse clinical observations.
c. Rat 2700 delivered and was euthanized on Day 21 of gestation.
d. Rat 2712 delivered and was euthanized on Day 21 of gestation.
* Significantly different from the control group value (p≤0.05).
** Significantly different from the control group value (p≤0.01).

TABLE 24 (PAGE 1): LITTER OBSERVATIONS (CAESAREAN-DELIVERED FETUSES) - SUMMARY - FEMALE RATS

GROUP		1	2	3	4
TEST MATERIAL		CONTROL ARTICLE	rAvPAL-PEG	rAvPAL-PEG	rAvPAL-PEG
DOSE LEVEL (MG/KG/DAY)a		0	2	8	20
LITTERS WITH ONE OR MORE LIVE FETUSES	N	24	24	23	21
IMPLANTATIONS	MEAN±S.D.	15.8 ± 1.5	14.4 ± 2.5	14.3 ± 1.9*	13.6 ± 2.5**
LIVE FETUSES	N	355	326	306	269
	MEAN±S.D.	14.8 ± 1.7	13.6 ± 2.8	13.3 ± 2.0	12.8 ± 2.6*
LIVE MALE FETUSES	N	165	162	157	147
% LIVE MALE FETUSES/LITTER	MEAN±S.D.	46.7 ± 12.1	49.6 ± 15.5	51.5 ± 11.7	54.1 ± 15.9
LIVE FETAL BODY WEIGHTS (GRAMS)/LITTER	MEAN±S.D.	5.57 ± 0.29 [23]b	5.75 ± 0.31	5.79 ± 0.32	5.09 ± 0.60**
MALE FETUSES	MEAN±S.D.	5.70 ± 0.31 [23]b	5.95 ± 0.34	5.95 ± 0.31*	5.23 ± 0.59**
FEMALE FETUSES	MEAN±S.D.	5.45 ± 0.27 [23]b	5.54 ± 0.36	5.62 ± 0.34	4.91 ± 0.67**
% DEAD OR RESORBED CONCEPTUSES/LITTER	MEAN±S.D.	6.1 ± 6.3	5.8 ± 10.6	6.6 ± 7.7	5.4 ± 8.8

[ ] = NUMBER OF VALUES AVERAGED
a. Dose administration occurred once daily beginning 28 days before cohabitation, during cohabitation and continued until Day 17 of gestation.
b. Excludes values for litter 2634 that was weighed after evisceration.
* Significantly different from the control group value (p≤0.05).
** Significantly different from the control group value (p≤0.01).

Fetal Examinations

There was a dose-dependent increase in litters (L) with abnormalities (8.3%, 29.2%, 43.5% and 66.7% in the control, 2, 8, and 20 mg/kg/day groups, respectively). The number of fetuses with any abnormality was 3 (0.8%), 9 (2.7%), 13 (4.2%) and 36 (13.4%) in the control, 2, 8, and 20 mg/kg/day groups, respectively.

TABLE 25 (PAGE 1): FETAL ALTERATIONS - CAESAREAN-DELIVERED LIVE FETUSES (DAY 21 OF GESTATION) - SUMMARY - FEMALE RATS

GROUP		1	2	3	4
TEST MATERIAL		CONTROL ARTICLE	rAvPAL-PEG	rAvPAL-PEG	rAvPAL-PEG
DOSE LEVEL (MG/KG/DAY)a		0	2	8	20
LITTERS EVALUATED	N	24	24	23	21
LITTERS WITH LIVE FETUS(ES)	N	24	24	23	21
FETUSES EVALUATED	N	355	326	306	269
LIVE	N	355	326	306	269
LITTERS WITH FETUSES WITH ANY ALTERATION OBSERVED	N(%)	2(8.3)	7(29.2)	10(43.5)**	14(66.7)**
FETUSES WITH ANY ALTERATION OBSERVED	N(%)	3(0.8)	9(2.8)	13(4.2)	36(13.4)**
% FETUSES WITH ANY ALTERATION/LITTER	MEAN±S.D.	0.8 ± 2.7	2.7 ± 5.3	4.4 ± 5.9*	13.0 ± 14.8**

a. Dose administration occurred once daily beginning 28 days before cohabitation, during cohabitation and continued until Day 17 of gestation.
* Significantly different from the control group value (p≤0.05).
** Significantly different from the control group value (p≤0.01).

rAvPAL-PEG at 8 and 20 mg/kg/day increased or significantly increased the number of litters, fetuses, and percentage of fetuses per litter with alterations. The significant increase in number of fetuses with abnormalities in the 8 mg/kg/day group was due to small increases in variations that included cervical ribs (4F (2.5%)/4L (17.4%)), bifid centra of lumbar and thoracic vertebrae (3F (1.8%)/3L (13%)) and incompletely ossified ribs (2F (1.2%)/2L (8.7%)). Increased variations in fetuses and/or litters in the 20 mg/kg/day group included significant increases in large sutures (nasal-frontal, 21F

(15.1%)/9L (42.8%); p ≤ 0.01), and incomplete ossification in squamosal bones (14F (10.1%)/8L (38.1%); p ≤ 0.01), frontal bones (22F (15.8%)/9L (42.8%); p ≤ 0.01), lumbar vertebra arch (3F (2.2%)/2L (9.5%)), ribs (7F (5%)/6L (28.6); p ≤ 0.01), summarization of ischium (9F (6.5%)/5L (23.8%); p ≤ 0.01) and pubis (7F/ (5%)/5L (23.8%); p ≤ 0.01). The increase in incomplete ossification in the 20 mg/kg/day group may be attributed to maternal toxicity.

Malformations observed in the treatment groups included microphthalmia (1F in the low-dose group), distended aorta and constricted pulmonary artery (1F in the high-dose group), 4 sacral vertebra, sacral hemivertebra and 12 rib pairs (1F in the mid-dose group), and split ribs (1F in the mid-dose group and 1F in the high-dose group). The incidence of malformations was low and was not dose-dependent. Thus, there were no treatment-related malformations.

Gross Pathology

There were no treatment-related findings.

Reproductive Organ Weights

Males:

rAvPAL-PEG at 20 mg/kg/day resulted in significant decreases in absolute reproductive organ weights [epididymides left (↓6.8%) or right (↓8.5%), cauda epididymis left (↓9.1%), seminal vesicles with fluid (↓15.5%) or without fluid (↓10.5%), and prostate (↓13.9%)]. However, these organ weights relative to body weight were comparable to the control values.

Females:

The absolute weight of the left ovary was reduced in the 20 mg/kg/day dose group, which corresponded to reductions in terminal body weight. However, the ovary weight relative to body weight was not affected by treatment.

Anti-rAvPAL IgG

For the 2, 8, and 20 mg/kg/day groups, 13/25, 5/25, and 4/25 male animals, respectively, developed anti-PAL IgG antibodies. For the 2, 8, and 20 mg/kg/day groups, 10/28, 0/25, and 0/25 female animals, respectively, developed anti-PAL IgG antibodies.

Hematology and Clinical Chemistry

As shown in the tables below (taken from the study report), rAvPAL-PEG produced ≥ 20% changes of some parameters of hematology and clinical chemistry. These

changes were dose-dependent and of similar magnitude in both sexes. Thus, these changes were treatment-related.

Table 54: Summary of Test Article-Related Hematology Parameters in Males

Parameter (Units)	Time Point	2 mg/kg/day	8 mg/kg/day	20 mg/kg/day
Reticuloyctes (10^9/L)	DS 1	+19.7	-4.5	+3.0
	Terminal	-1.1	+1.5	-15.5*
Eosinophils (10^3/cmm)	DS 1	+7.6	+10.5	+99.6
	Terminal	+0.5	-7.1	-45.3*
Basophils (10^3/cmm)	DS 1	+22.2	+20.0	+15.6
	Terminal	-10.0	-21.0	-27.0*

All values presented as percent of control group value.
DS = Study Day
Terminal = DS 78, 79, 80 or 81
* Significantly different from the control group value a $p \leq 0.05$

Table 55: Summary of Test Article-Related Clinical Chemistry Parameters in Males

Parameter (Units)	Time Point	2 mg/kg/day	8 mg/kg/day	20 mg/kg/day
ALT (IU/L)	DS 1	+6.1	+2.2	+2.4
	Terminal	-4.2	-15.0*	-23.4*
Serum GGT (IU/L)	DS 1	+13	+13	+13
	Terminal	+108.3	+204.3	+627.3*
Triglyceride (mg/dL)	DS 1	+1.1	+13.4	-8.4
	Terminal	-11.0	-30.2	-54.5*

All values presented as percent of control group value.
DS = Study Day
Terminal = DS 78, 79, 80 or 81
* Significantly different from the control group value a $p \leq 0.05$

Table 56: Summary of Test Article-Related Hematology Parameters in F0 Females

Parameter (Units)	Time Point	2 mg/kg/day	8 mg/kg/day	20 mg/kg/day
Reticuloyctes (10^9/L)	DS 1	-18.0	-25.9	-40.7*
	DG 21	+2.9	+4.7	-38.1*
Eosinphils (10^3/cmm)	DS 1	-8.5	+23.8	+16.7
	DG 21	+0.7	-7.1	-57.7*

All values presented as percent of control group value.
DS = Study Day; DG = Day of Gestation
* Significantly different from the control group value a $p \leq 0.05$

Table 57: Summary of Test Article-Related Clinical Chemistry Parameters in F0 Females

Parameter (Units)	Time Point	2 mg/kg/day	8 mg/kg/day	20 mg/kg/day
ALT (IU/L)	DS 1	+16.2	+9.9	-6.3
	DG 21	+1.5	-0.6	-18.1*
Alkaline Phosphatase (IU/L)	DS 1	+6.3	-6.4	-9.8
	DG 21	+5.5	+6.7	-31.3*
Cholesterol (mg/dL)	DS 1	+0.7	+0.5	-3.5
	DG 21	+2.1	-4.6	-19.3*
Total Bilirubin (mg/dL)	DS 1	-1.6	+3.6	-11.5
	DG 21	-9.0	-8.4	-20.4*
Triglyceride (mg/dL)	DS 1	+0.3	-22.1	-10.7
	DG 21	-13.6	-35.7*	-36.0*

All values presented as percent of control group value.
DS = Study Day; DG = Day of Gestation
* Significantly different from the control group value a $p \leq 0.05$

Dosing Solution Analysis

The concentrations of all dose formulation samples were within the acceptable range (± 15% of nominal concentrations) and all dose formulations were stable following storage at 2°C to 8°C for 8 days.

9.2 Embryonic Fetal Development

Study title: Embryo-fetal Development Study of rAvPAL-PEG Administered by Subcutaneous Injection in Rabbits

Study no.:	BMN165-12-036
Study report location:	N/A
Conducting laboratory and location:	(b) (4)
Date of study initiation:	November 1, 2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	2011-165-15-111511; 100%

Key Study Findings

1. rAvPAL-PEG at 5 mg /kg/day produced a high incidence of external malformations of the head, body, and limbs, and multiple malformations in visceral organs and all regions of the skeletal system. Although the dose which produced malformations also caused clear signs of maternal toxicity (e.g. abortion and premature death in

8% of rabbits at 5 mg/kg/day, marked impairment of weight gain and food consumption in the surviving females), the malformations are not considered to be secondary to maternal toxicity based on their severity and high incidence (i.e.> 50% of fetuses and litters).

2. rAvPAL-PEG at 5 mg/kg/day produced a significant increase in late resorptions, post-implantation loss, and the number of does with any resorptions. Reductions in total live fetal body weight, male and female body weight, and the number of live male fetuses also occurred at 5 mg/kg/day.

3. At the lowest dose tested (2 mg/kg/day), weight gain and food consumption were reduced, but there was no increase in malformations.

4. The observed reduction in weight gain during days 7-29 of gestation was 23% and 59% in the 2 and 5 mg/kg/day groups, respectively. These values represent the mean reduction observed in the three treatment periods used in this study (i.e. GD 7-12, 11-16 and 15-20).

5. During treatment (days 7-20 of gestation), blood phenylalanine levels in the 2 and 5 mg/kg/day groups were decreased by an average of 90% and 99%, respectively.

6. rAvPAL-PEG was detected in fetal plasma during the treatment periods used in this study (i.e. GD 7-12, 11-16 and 15-20).

7. The NOAEL for embryo-fetal developmental toxicity was 2 mg/kg/day. The maternal NOAEL was not determined (< 2 mg/kg/day), due to the reductions in weight gain and food consumption in the 2 mg/kg/day group.

Methods

Doses:	0 (vehicle), 2, and 5 mg/kg
Frequency of dosing:	Once daily
Dose volume:	2 ml/kg
Route of administration:	subcutaneous; injection sites were rotated among four areas in the scapular region
Formulation/Vehicle:	rAvPAL-Peg was dissolved in vehicle solution (10 mM TRIS, 135 mM sodium chloride, 1 mM trans-cinnamic acid, 1 mM ammonia), pH 7.0 ± 0.5) at concentrations of 1 and 2.5 mg/mL
Species/Strain:	New Zealand white [Hra:(NZW)SPF] rabbits
Number/Sex/Group:	20 females
Satellite groups:	3 females for TK
Study design:	Study design is shown in the table below (taken from the study report).
Deviation from study protocol:	Deviations had no impact on data integrity or

interpretation of data.

As shown in the table below (taken from the study report), pregnant females were treated with rAvPAL-PEG using a divided dosing regimen (gestation days (GD) 7 to 12, GD 11 to 16, or GD 15 to 20). The use of divided dosing periods during organogenesis (GD 7 to 20) was needed to assure that adequate test-article exposure occurred throughout organogenesis, since a decline in exposure to test-article was observed beyond 7 days of dosing in a dose range-finding study (study report #: BioMarin BMN165-11-027).

Table 58: Experimental Design for Embryonic Fetal Development in Rabbits

Group No.	Test Material	Dose Level (mg/kg)	Dose Period (DGs)	Concentration (mg/mL)	Dose Volume (mL/kg)	No. of Main Study Animals	No. of Toxicokinetic Animals
1	Control Article	0	7-20	0	2	20	3
2	rAvPAL-PEG	2	7-12	1	2	20	3
	Control Article[a]	0	13-20	0	2		
3	Control Article[b]	0	7-10, 17-20	0	2	20	3
	rAvPAL-PEG	2	11-16	1	2		
4	Control Article	0	7-14	0	2	20	3
	rAvPAL-PEG	2	15-20	1	2		
5	rAvPAL-PEG	5	7-12	2.5	2	20	3
	Control Article[a]	0	13-20	0	2		
6	Control Article[b]	0	7-10, 17-20	0	2	20	3
	rAvPAL-PEG	5	11-16	2.5	2		
7	Control Article	0	7-14	0	2	20	3
	rAvPAL-PEG	5	15-20	2.5	2		

[a] Toxicokinetic rabbits in Groups 2 and 5 were not administered the control article on Days 19 or 20 of presumed gestation (DGs 19 or 20) as these rabbits were euthanized on DG 19.

[b] Toxicokinetic rabbits in Groups 3 and 6 were not administered the control article on DGs 19 or 20 as these rabbits were euthanized on DG 19.

DG - Day of Presumed Gestation

In the dose range-finding study, pregnant rabbits were treated with test-article at doses of 2, 5, or 20 mg/kg/day via SC injection during GD 7-20 using a divided dosing-period plan, as shown in the table below (taken from the dose range-finding study report).

Table 59: Study Design for Dose Range-Finding Study in Rabbits

Experimental Design - Part A

Group No.	Test Material	Dose Level (mg/kg)	Concentration (mg/mL)	Dose Volume (mL/kg)	No. of Main Study Animals	No. of Toxicokinetic Animals
Dose Administration on Days 7 to 20 of Presumed Gestation						
1	Control Article	0	0	2	10	3
2	rAvPAL-PEG	2	1	2	10	3
Dose Administration on Days 7 to 14 of Presumed Gestation						
3	rAvPAL-PEG	2	1	2	10	3
Dose Administration on Days 13 to 20 of Presumed Gestation						
4	rAvPAL-PEG	2	1	2	10	3

Experimental Design - Part B

Group No.	Test Material	Dose Level (mg/kg)	Concentration (mg/mL)	Dose Volume (mL/kg)	No. of Main Study Animals	No. of Toxicokinetic Animals
Dose Administration on Days 7 to 20 of Presumed Gestation						
5	rAvPAL-PEG	20	10	2	10	3
Dose Administration on Days 11 to 16 of Presumed Gestation						
6	rAvPAL-PEG	20	10	2	10	3
Dose Administration on Days 15 to 20 of Presumed Gestation						
7	rAvPAL-PEG	20	10	2	10	3

Experimental Design - Part C

Group No.	Test Material	Dose Level (mg/kg)	Concentration (mg/mL)	Dose Volume (mL/kg)	No. of Main Study Animals	No. of Toxicokinetic Animals
Dose Administration on Days 7 to 20 of Presumed Gestation						
8	rAvPAL-PEG	5	2.5	2	10	3
Dose Administration on Days 11 to 16 of Presumed Gestation						
9	rAvPAL-PEG	5	2.5	2	10	3
Dose Administration on Days 15 to 20 of Presumed Gestation						
10	rAvPAL-PEG	5	2.5	2	10	3

Following 2 or 3 doses, rAvPAL-PEG at 2 or 5 mg/kg/day generally produced suppression of phenylalanine (Phe) levels to below the limit of quantitation (LOQ: 1 µM) up to 6 days at 5 mg/kg/day. However, exposure to test-article declined after more than 7 days of dosing of 2 and 5 mg/kg/day. rAvPAL-PEG at 20 mg/kg/day produced suppression of Phe levels to below the LOQ for the entire dosing period (GD 7 to 20), and the suppressed Phe level was not reversed on the last sampling day (GD 25) following dosing. Administration of 20 mg/kg/day of rAvPAL-PEG caused severe maternal toxicity (e.g. anorexia with concurrent weight loss, dehydration, hypoactivity, liquid/mucoid feces, hyperpnea, bradypnea, and hunched posture), which resulted in early termination of this group. Based on the dose range-finding study, the Sponsor

concluded that the 5 mg/kg/day dose produced minimal maternal toxicity and some developmental toxicity (embryo-fetal death, reductions in fetal weight and/or an increase in fetal alterations), and that 2 mg/kg/day was expected to be the NOAEL in the definitive study. Thus, the selected doses for the embryo-fetal developmental study in rabbits were 2 and 5 mg/kg/day. The TK data from the dose-ranging study in rabbits is summarized in the tables below (taken from the study report).

Text Table 24
Mean TK Parameters for rAvPAL-PEG Following Once-Daily SC Injections of 2 mg/kg/day to Time-Mated Female Rabbits (Groups 2 through 4)

Group	Day of Gestation (DG)	$AUC_{0\text{-}24\ hrs}$ (ng-hr/mL)		$AUC_{0\text{-}t}$ (ng-hr/mL)		C_{max} (ng/mL)[a]		T_{max} (hours)[a]		$C_{24\ hrs}$ (ng/mL)		AR	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
2	7	246499	60972	246499	60972	28933	7557	24	0	28933	7557	–	–
	20[b]	–	–	–	–	–	–	–	–	–	–	–	–
3	7	255153	53523	255153	53523	28333	11140	24.0	0.0	28333	11140	–	–
	14	2497	2689	2497	2689	287	206	8.0	4.0	136	107	0.012	0.015
4	13	236841	78413	236841	78413	23767	4277	24.0	0.0	23767	4277	–	–
	20	31333	15127	39087	14558	6447	7322	4.0	6.9	778	697	0.160	0.134

a: C_{max} and T_{max} were based on sample collections that occurred predose through 24 hours postdose.
b: Plasma concentrations of rAvPAL-PEG were all BLQ (<50 ng/mL) for the last dose on DG20 for Group 2.
AR: accumulation ratio [$(AUC_{0\text{-}24\ hrs,\ Last\ Dose})/AUC_{0\text{-}24\ hrs,\ First\ Dose}$]; SD: standard deviation.

Text Table 25
Mean TK Parameters for rAvPAL-PEG Following Once-Daily SC Injections of 20 mg/kg/day to Time-Mated Female Rabbits (Groups 5 through 7)

Group	Day of Gestation (DG)	$AUC_{0\text{-}24\ hrs}$ (ng-hr/mL)		$AUC_{0\text{-}t}$ (ng-hr/mL)		C_{max} (ng/mL)[a]		T_{max} (hours)[a]		$C_{24\ hrs}$ (ng/mL)		AR	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
5	7	1540675	835831	1540675	835831	175333	74501	24.0	0.0	175333	74501	–	–
	20[b]	–	–	–	–	–	–	–	–	–	–	–	–
6	11	902140	171863	902140	171863	121700	23381	24.0	0.0	121700	23381	–	–
	16	42746667	5205856	524800000	71079460	2270000	357631	18.67	9.24	2136667	559673	47.8	3.68
7	15	821924	135163	821924	135163	125000	16371	24.0	0.0	125000	16371	–	–
	20	32163333	6820076	149335333	22955192	1800000	376431	2.67	2.31	1626667	378726	39.6	9.56

a: C_{max} and T_{max} were based on sample collections that occurred predose through 24 hours postdose.
b: Due to unscheduled terminal procedures occurring on DG18 for all animals in Group 5, dosing and collecting samples after DG18 were not possible.
AR: accumulation ratio [$(AUC_{0\text{-}24\ hrs,\ Last\ Dose})/AUC_{0\text{-}24\ hrs,\ First\ Dose}$]; SD: standard deviation.

Text Table 26
Mean TK Parameters for rAvPAL-PEG Following Once-Daily SC Injections of 5 mg/kg/day to Time-Mated Female Rabbits (Groups 8 through 10)

Group	Day of Gestation (DG)	$AUC_{0\text{-}24\ hrs}$ (ng-hr/mL)		$AUC_{0\text{-}t}$ (ng-hr/mL)		C_{max} (ng/mL)[a]		T_{max} (hours)[a]		$C_{24\ hrs}$ (ng/mL)		AR	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
8	7	667767	233542	667767	233542	77667	33307	22.0	3.5	77667	33307	–	–
	20	872383	715862	894271	727892	64660	51990	2.7	4.6	10857	9824	1.30	1.23
9	11	635113	148866	635113	148866	59633	22107	22.0	3.5	58133	24236	–	–
	16	13367667	1790769	13367667	1790769	767667	86234	10.7	12.2	618667	266010	21.7	4.83
10	15	583740	215940	635113	148866	59000	11004	22.0	3.5	56467	8659	–	–
	20	12697333	4265097	61481104	60820245	744000	247586	10.0	7.2	477333	97762	22.1	3.92

a: C_{max} and T_{max} were based on sample collections that occurred predose through 24 hours postdose.
AR: accumulation ratio [$(AUC_{0\text{-}24\ hrs,\ Last\ Dose})/AUC_{0\text{-}24\ hrs,\ First\ Dose}$]; SD: standard deviation.

Observations and Results

Mortality

Drug-related deaths occurred in the groups treated with 5 mg/kg/day (see table below under Clinical Signs).

Clinical Signs

A total of 1, 0, 0, 1, 1, and 2 does in groups 2, 3, 4, 5, 6 and 7, respectively, aborted and one doe in group 7 delivered on GD 29. The single delivery on GD 29 in the 5 mg/kg/day groups (# 5-7) and a single abortion in the 2 mg/kg/day groups (# 2-4) are within the historical range (early delivery: 1/571; abortion: 3/571 does). Therefore, the early delivery in the 5 mg/kg/day groups (# 5-7) and abortion in the 2 mg/kg/day groups (# 2-4) were not considered as related to treatment. However, rAvPAL-PEG at 5 mg/kg/day increased the incidence of abortion (historical control of abortion: 3/571 does; abortion in the 5 mg/kg/day groups (# 5-7): 4/60 does). Thus, the incidence of abortion in the 5 mg/kg/day group was treatment-related. Information for does and uterine contents is summarized in the tables below (taken from the study report).

Table 60: Summary of Mortality and Clinical Signs

Rabbit Number	3929	3988	4002	4030	4031	4038
Group	2	5	6	7	7	7
Dose (mg/kg)	2	5	5	5	5	5
Scheduled rAvPAL-PEG Dose Period	DGs 7-12	DGs 7-12	DGs 11-16	DGs 15-20	DGs 15-20	DGs 15-20
Doses Administered	6	6	6	6	6	6
Day of Death	DG 28	DG 28	DG 22	DG 29	DG 26	DG 26
Mode of Death	AE	AE	AE	DE	AE	AE
Timing of Death Relative to Last Dose	16 days	16 days	6 days	9 days	6 days	6 days
Clinical Observations (DGs)						
No feces in cage pan	15,18,21	-	-	-	-	-
Scant feces	18,21,24, 27, 28	14,15,18,21,23,24	11,14,17,18,20-22	18,19,21,24,26-28	17,24,25	18,21,24
Soft or liquid feces	-	-	-	21	17	-
Injection Site: Purple	21-24	10-11	-	15,16	13-16	-
Thin body condition	24, 26-28	20-25	17,19-22	19,21,24,27-29	18-26	21,24-26
Dehydration - Mild	27	-	20-22	25-29	23,24	-
Dehydration - Moderate	28	-	20	-	25,26	-
Red perivaginal substance on fur	28	-	22	-	-	-
Red perioral substance on fur	28	-	-	-	-	-
Excess salivation - slight to moderate				20,21,22-24	-	
Red substance in cage pan or liner	28	28	-	28,29	26	26
Yellow perinasal substance	-	17-19	20	20-24	-	-
Yellow perioral substance	-	17-19	-	20,23,25-27	-	-
Ptosis	-	-	20		-	-
Bradypnea	-	-	20		-	-
Ungroomed coat	-	-	20	19,21,23,24,26-29	20,23-25	23,24
Fecal-stained fur	-	-	20	19,21,24	20,23-25	24
Yellow fur (nose, face, mouth and/or neck)				21,24	24	24
Red perivaginal substance	-	-	-	-	24-25	-
Red mixed with urine substance on liner	-	-	-	-	24-25	-
Decreased motor activity	-	-	-	29	-	-
Cold to touch	-	-	-	29	-	-

DG - Day of Gestation; AE – aborted and euthanized; DE – delivered and euthanized

Table 61: Summary of Mortality and Clinical Signs (Continued)

Rabbit Number	3929	3988	4002	4030	4031	4038
Group	2	5	6	7	7	7
Dose (mg/kg)	2	5	5	5	5	5
Scheduled rAvPAL-PEG Dose Period	DGs 7-12	DGs 7-12	DGs 11-16	DGs 15-20	DGs 15-20	DGs 15-20
Doses Administered	6	6	6	6	6	6
Day of Death	DG 28	DG 28	DG 22	DG 29	DG 26	DG 26
Mode of Death	AE	AE	AE	DE	AE	AE
Timing of Death Relative to Last Dose	16 days	16 days	6 days	9 days	6 days	6 days
Body Weights						
Change from DG 7 through Day of Death	-0.79 kg	-0.39 kg	-0.61 kg	-0.69 kg	-0.94 kg	-0.42 kg
Food Consumption						
Trend during study period	Severely reduced	Severely reduced	Severely reduced	Severely reduced	Severely reduced	Severely reduced
Necropsy Observations						
All tissues appeared normal	X	X	X	X	X	X
Uterine Contents	9DF	1DF, 7LR, 2ER	5LR	9DF, 2LR	5DF, 4 empty implants (presumed cannibalized)	2DF, 3LF, 5LR

DG - Day of Gestation; LF – Live Fetus; DF - Dead Fetus; ER - Early resorptions; LR - Late resorption

The does treated with 2 mg/kg/day on GDs 11-16 showed an increased incidence of scant feces (10/20 does vs. 0/20 does in the control group) and reduced feces (5/20 vs. 0/20 does in the control group).

The following adverse clinical signs were observed in does receiving rAvPAL-PEG at 5 mg/kg/day (groups 5-7): scant/reduced feces, soft or liquid feces, thin body condition, sparse hair coat, mild and/or moderate dehydration, yellow, green, or brown perioral or peri-nasal substance, excess salivation, yellow or brown substance on the neck, around the mouth, face, forelimbs and/or forepaws, red and/or brown substance on the facial, peri-vaginal and/or genital fur, urine staining, no feces in the cage pan, red and/or dark pink on the neck, chin, eye lids, around the mouth, and periorbital membrane, ptosis, decreased motor activity, lacrimation, bradypnea, and ungroomed coat.

Body Weight

Although significant differences in body weight were observed in the drug-treated groups, the baseline bodyweight of the control group (GD 0 and 7) was substantially lower than the baseline weights of the treatment groups. Therefore, the effects of rAvPAL-PEG on bodyweight cannot be evaluated.

Table 62: Maternal Body weight in Does during Gestation Days Following rAVPAL-PEG Treatment

Gestation days	Dose groups (mg/kg/day)						
	0	2			5		
	Gestation Days for Dosing						
	7-20	7-12	11-16	15-20	7-12	11-16	15-20
	Body weight (kg)						
0	3.22	3.59**	3.48*	3.34	3.6**	3.43*	3.32
7	3.33	3.68**	3.55*	3.5	3.68**	3.52*	3.46
8	3.37	3.73**	3.6*	3.53	3.74**	3.59*	3.48
9	3.4	3.75**	3.63*	3.56	3.77**	3.61*	3.51
10	3.43	3.76**	3.64*	3.57	3.76**	3.63*	3.52
11	3.43	3.77**	3.65*	3.6	3.76**	3.63*	3.54
12	3.46	3.76**	3.86*	3.63	3.7**	3.66*	3.57
13	3.49	3.75**	3.7	3.65	3.64*	3.68	3.6
14	3.52	3.77**	3.74	3.69	3.57	3.7	3.62
15	3.52	3.78**	3.76*	3.7	3.49	3.71	3.62
16	3.55	3.8**	3.76	3.72	3.46	3.66	3.63
17	3.58	3.83**	3.76	3.75	3.48	3.62	3.63
18	3.59	3.84**	3.74	3.76	3.53	3.55	3.64
19	3.62	3.85**	3.76	3.77	3.56	3.49	3.64
20	3.63	3.89**	3.78	3.78	3.60	3.47	3.6
29	3.75	3.99**	3.88	3.83	3.83	3.67	3.68

* Significantly different from the control group value (p≤0.05). ** Significantly different from the control group value (p≤0.01).

rAvPAL-PEG at 2 mg/kg/day resulted in a significant decrease in maternal body weight gain during GD 7-12 (↓56%), but had no effects on maternal body weight gain during the GD 11-16 or 15-20. However, all groups treated with rAvPAL-PEG at 5 mg/kg/day

(GD 7-12, 11-16, and 15-20) showed a marked reduction of weight gain, or weight loss, as shown in the table below.

Table 63: Maternal Body Weight Changes in Does during Gestation Days with rAVPAL-PEG Treatment

Gestation days	Dose groups (mg/kg/day)						
	0	2			5		
	Gestation Days for Dosing						
	7-20	7-12	11-16	15-20	7-12	11-16	15-20
	Body weight change (kg)						
0-7	+0.11	+0.09	+0.07	+0.16	+0.09	+0.09	+0.14
7-13	+0.16	+0.07*	-	-	-0.04**	-	-
14-17	+0.06	-	+0.03	-	-	-0.08**	-
11-17	+0.15	-	+0.11	-	-	-0.01**	-
15-21	+0.14	-	-	+0.11	-	-	-0.05**
17-21	+0.08	-	+0.05	+0.06	-	-0.12**	-0.06**
7-29	+0.42	+0.31	+0.33	+0.33	+0.15**	+0.15**	+0.22**
0-29	+0.53	+0.4	+0.4	+0.49	+0.23**	+0.24**	+0.36

* Significantly different from the control group value (p≤0.05).
** Significantly different from the control group value (p≤0.01).
-: Not applicable.

All treatment groups showed major reductions in weight gain during GD 7-29. The observed reductions in weight gain (GD 7-29) were 26%, 21%, 21%, 64%, 64%, and 48% in groups 2, 3, 4, 5, 6, and 7, respectively

Feed Consumption

rAvPAL-PEG at 2 mg/kg/day produced in a significant decrease in absolute food consumption (g/day) during the treatment period of GD 7-12 (↓12.5%), but had no effects on absolute food consumption during the treatment periods of GD 11-16 or 15-20. However, does given 5 mg/kg/day during the three treatment periods had significantly reduced absolute food consumption (↓up to 50.5%) as shown in the table

below. Overall, the reduced absolute food consumption lasted the entire gestation period (see table below).

Table 64: Maternal Absolute Food Consumption in Does during Gestation Days Following rAVPAL-PEG Treatment

Gestation days	Dose groups (mg/kg/day)						
	0	2			5		
	Dosing days						
	7-20	7-12	11-16	15-20	7-12	11-16	15-20
	Absolute food consumption (g/day)						
7-10	171.9	163.3	159.7	174.5	158.7	166.3	160.3
7-11	171.2	-	159.4	-	-	164.9	-
7-13	168.6	147.6* (↓12.5%)	-	-	117.7** (↓30.2%)	-	-
10-14	161.8	129.1** (↓20.2%)	149.4	167.2	63.4**	148.9	148.7
14-17	167.2	125.4**	140.9	158.8	32.7**	82.7** (↓50.5%)	131.5
11-17	163.3	-	143.6	-	-	113.7** (↓30.4%)	-
15-21	167.5	-	-	157.4	-	-	100.4** (↓40.1%)
17-21	167.4	148.4	121.3* (↓27.6%)	157.8	108.2** (↓35.4%)	43.4** (↓74.1)%	86.7** (↓48.2%)
7-21	166.7	140.9** (↓23%)	141.8	164.3	89.8** (↓46.1%)	108.3** (↓35%)	128.3

* Significantly different from the control group value (p≤0.05).
** Significantly different from the control group value (p≤0.01).

- Not applicable.

The trend of changes in relative food consumption (g/kg/day) in does treated with 2 and 5 mg/kg/day was same as the absolute food consumption during the treatment period.

Toxicokinetics

Overall, plasma concentrations of rAvPAL-PEG at the three time-points measured in each treatment period (GD 7-12, 11-16, and 15-20) was dose-proportional. rAvPAL-PEG was present in the fetal plasma after treatment during each treatment period. Plasma concentrations of test article in does and fetuses are summarized in the tables below (taken from the study report).

Text Table 19
Plasma Concentrations of rAvPAL-PEG (ng/mL) after Once-Daily Subcutaneous Injections of rAvPAL-PEG to Female New Zealand White Rabbits during Gestation (DG 7-12)

Group	Treatment[a]	Dose Level (mg/kg/day)	Dose Period (DG)	Animal Number	DG Sampling Time Points			
					8 (0 hr)	10 (0 hr)	13 (24 hr)	19 (Fetal)
2	rAvPAL-PEG	2[b]	7-12[b]	4044[b]	23300	NS	9710[b,c]	419
				4045	24000	NS	161000	186
				4046	23400	NS	27200	232
				Mean	23567	NA	94100	279
				SD	379	NA	94611	123
				%CV	1.6	NA	100.5	44.2
5	rAvPAL-PEG	5	7-12	4053	46900	197000	550000	923
				4054	NS	NS	466000	746
				4055	73800	1100	300000	1810
				Mean	60350	99050	438667	1160
				SD	19021	138522	127222	570
				%CV	31.5	139.9	29.0	49.2

Note: Maternal TK rabbits were euthanized on DG19, at which time a fetal sample was obtained for analysis.
a: Animals were given control article on DG13-18.
b: As a deviation to the protocol, Animal 4044 (Group 2) inadvertently received rAvPAL-PEG at 5 mg/kg only on DG12; the planned 2-mg/kg dose was given on DG7-11.
c: Value not included in descriptive statistics and graphing due to protocol deviation.
CV: Coefficient of variation = (SD / Mean) x 100%.
DG: Day of presumed gestation.
hr: Hours postdose; "24 hr" collections occurred approximately 24 hours after the last dose of rAvPAL-PEG was administered, and "0 hr" samples were collected predose.
NA: Not applicable.
NS: No sample.
SD: Standard deviation.

Text Table 21
Plasma Concentrations of rAvPAL-PEG (ng/mL) after Once-Daily Subcutaneous Injections of rAvPAL-PEG to Female New Zealand White Rabbits during Gestation (DG 11-16)

Group	Treatment[a]	Dose Level (mg/kg/day)	Dose Period (DG)	Animal Number	DG Sampling Time Points			
					12 (0 hr)	14 (0 hr)	17 (24 hr)	19 (Fetal)
3	rAvPAL-PEG	2	11-16	4047	20700	134000	78600	342
				4048	1960	132000	59900	286
				4049	1520	87300	14700	322
				Mean	8060	117767	51067	317
				SD	10949	26404	32853	28
				%CV	135.8	22.4	64.3	9.0
6	rAvPAL-PEG	5	11-16	4056	1740	214000	262000	1450
				4057	1250	239000	242000	1080
				4058	1360	149000	397000	1190
				Mean	1450	200667	300333	1240
				SD	257	46458	84311	190
				%CV	17.7	23.2	28.1	15.3

Note: Maternal TK rabbits were euthanized on DG19, at which time a fetal sample was obtained for analysis.
a: Animals were given control article on DG7-10 and DG17-18.
CV: Coefficient of variation = (SD / Mean) x 100%.
DG: Day of presumed gestation.
hr: Hours postdose; "24 hr" collections occurred approximately 24 hours after the last dose of rAvPAL-PEG was administered, and "0 hr" samples were collected predose.
SD: Standard deviation.

Text Table 23
Plasma Concentrations of rAvPAL-PEG (ng/mL) after Once-Daily Subcutaneous Injections of rAvPAL-PEG to Female New Zealand White Rabbits during Gestation (DG 15-20)

Group	Treatment[a]	Dose Level (mg/kg/day)	Dose Period (DG)	Animal Number	DG Sampling Time Points			
					16 (0 hr)	18 (0 hr)	21 (24 hr)	21 (Fetal)
4	rAvPAL-PEG	2	15-20	4050	19600	129000	168000	NS
				4051	19000	70000	95800	612
				4052	22200	89800	30300	540
				Mean	20267	96267	98033	576
				SD	1701	30027	68877	51
				%CV	8.4	31.2	70.3	8.8
7	rAvPAL-PEG	5	15-20	4059	50200	203000	413000	305
				4060	57100	275000	440000	989
				4061	54000	239000	358000	408
				Mean	53767	239000	403667	567
				SD	3456	36000	41789	369
				%CV	6.4	15.1	10.4	65.0

Note: Maternal TK rabbits were euthanized on DG21, at which time a fetal sample was obtained for analysis.
a: Animals were given control article on DG7-14.
CV: Coefficient of variation = (SD / Mean) x 100%.
DG: Day of presumed gestation.
hr: Hours postdose; "24 hr" collections occurred approximately 24 hours after the last dose of rAvPAL-PEG was administered, and "0 hr" samples were collected predose.
NS: No sample.
SD: Standard deviation.

Plasma phenylalanine (Phe) levels were measured three times during each treatment period. In does treated with 2 and 5 mg/kg/day rAvPAL-PEG (groups 2-4 and groups 5-7, respectively), Phe concentration was markedly reduced (2 mg/kg/day: 1.09-24.7 µM; 5 mg/kg/day: 2.82 – 5.37) or below the limit of quantitation (<1.00 µM). The average

reduction in plasma Phe at 2 and 5 mg/kg/day was 90% and 99%, respectively, relative to the mean control value of 52.5 µM.

Clinical Pathology

At 5 mg/kg/day, rAvPAL-PEG produced changes in hematology and clinical chemistry (AST and ALT), as shown in the tables below (taken from study report).

Table 65: Summary of Changes in Hematology Parameters

Parameter (Units)	Time Point (DG)	2 mg/kg (Group 2)	2 mg/kg (Group 4)	5 mg/kg (Group 5)	5 mg/kg/ (Group 7)
Reticulocytes (10^9/L)	20	-	-17.4*	-	-34.6*
Lymphocytes (10^3/cmm)	20	-	-23.6*	-	-27.0*
Monocytes (10^3/cmm)	7	+47.5	-	+69.2*	-
	15	-	+41.7	-	+178.9*
	20	-	+3.6	-	+65.6
Segmented Neutrophils (10^3/cmm)	20	-	+21.9	-	+66.7*
Eosinophils (10^3/cmm)	15	-	+30.2	-	+49.9*

All values presented as percent of control group value.
DG = Day of Gestation
* Significantly different from the control group value ($p < 0.05$).

Table 66: Summary of Changes in Clinical Chemistry Parameters

Parameter (Units)	Time Point (DG)	2 mg/kg/day (Group 2)	2 mg/kg/day (Group 4)	5 mg/kg/day (Group 5)	5 mg/kg/day (Group 7)
AST (IU/L)	20	-	-36.9	-	-41.3*
ALT (IU/L)	7	0.6	-	-20.4	-
	20	-	-21.5	-	-32.7*

All values presented as percent of control group value.
DG = Study Day
* Significantly different from the control group value ($p < 0.05$).

Dosing Solution Analysis

The concentrations of all dose formulations were within or equal to the acceptance criteria of ± 15% (individual values within or equal to ± 20%) of their theoretical concentrations.

Necropsy

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

rAvPAL-PEG at 2 mg/kg/day had no adverse effects on ovarian, uterine, or litter parameters as shown in the table below. The average weight of the female fetuses

from the 2 mg/kg/day does treated during GD 11-16 was significantly reduced compared to controls (↓8.9%).

Administration of rAvPAL-PEG at 5 mg/kg/day during GD 7-12, but not GD 11-16 or GD 15-20, significantly increased late resorptions, post-implantation loss, and the number of does with any resorptions as shown in the table below. rAvPAL-PEG at 5 mg/kg/day given during GD 7-12, 11-16, and 15-20 significantly reduced the total live fetal body weight (↓up to 38.5%), male body weight (↓up to 38.6%), and female body weight (↓36.7%), as shown in the table below. In addition, does treated with rAvPAL-PEG at 5 mg/kg/day during GD 11-16 had significantly reduced live male fetuses (↓29%).

Table 67: Cesarean Section Data (resorptions, implantation loss and does with any resorptions)

Parameters		Dose groups (mg/kg/day)						
		0	2			5		
		Dosing days	Dosing days			Dosing days		
		7-20	7-12	11-16	15-20	7-12	11-16	15-20
Rabbits tested	N	20	20	20	20	20	20	20
Pregnant	N (%)	20 (100)	20 (100)	19 (95)	20 (100)	20 (100)	20 (100)	18 (90)
Aborted and euthanized	N (%)		1(5)	0 (0)	0 (0)	1 (5)	1 (5)	2 (11.1)
Delivered and euthanized	N (%)	0	0	0	0	0	0	1 (5.6)
Rabbits pregnant and caesarean-section on day 29 of gestation	N	20	19	19	20	19	19	15
Resorptions	Mean ± SD	0.2 ± 0.4	0.2 ± 0.4	0.4 ± 0.6	0.4 ± 0.6	1.3 ± 1.2**	0.5 ± 0.9	0.3 ± 0.4
Late Resorptions	N Mean ± SD	2 0.1 ± 0.3	3 0.2 ± 0.4	3 0.2 ± 0.4	3 0.2 ± 0.5	18 0.9 ± 1.1*	6 0.3 ± 0.7	4 0.3 ± 0.4

% Post-implantation loss	Mean ± SD	2.2 ± 4.7	2.2 ± 4.3	4.8 ± 7.0	4.7 ± 8.8	14.5 ± 11.6**	6.2 ± 10.1	2.4 ± 4.2
Does with any resorptions	N (%)	4 (20.0)	4 (21.0)	7 (36.8)	6 (30)	14 (73.7)**	6 (31.6)	4 (26.7)

* Significantly different from the control group value (p≤0.05).
** Significantly different from the control group value (p≤0.01).

Table 68: Cesarean Section Delivered Fetuses (Litter Observations at 5 mg/kg/day)

Parameters		Dose groups (mg/kg/day)			
		0	5		
		Dosing days	Dosing days		
		7-20	7-12	11-16	15-20
Litters with one of more live fetuses	N	20	19	19	15
Implantations	Mean ± SD	9.0 ± 1.7	9.4 ± 1.9	8.9 ± 1.4	9.5 ± 1.7
Live fetuses	N Mean ± SD	177 8.8 ± 1.7	153 8.0 ± 1.9	159 8.4 ± 1.4	139 9.3 ± 1.5
Live male fetuses	N	91	73	58	70
% Live male Fetuses/litter	Mean ± SD	50.7 ± 18.5	45.4 ± 19.8	36.0 ± 16.3**	51.1 ± 15.9
Live fetal body weights (g)	Mean ± SD	43.65 ± 6.64	28.98 ± 7.48**	26.86 ± 5.54**	28.22 ± 5.76**
Male fetuses	Mean ± SD	44.38 ± 6.89	28.85 ± 8.26** [18]a	27.24 ± 5.70**	28.63 ± 6.12**
Female fetuses	Mean ± SD	43.01 ± 6.38	28.58 ± 7.58**	26.71 ± 5.66**	27.71 ± 5.87**
% Dead or resorbed conceptuses/litter	Mean ± SD	2.2 ± 4.7	14.5 ± 11.6**	6.2 ± 10.1	2.4 ± 4.2

[ ] = NUMBER OF VALUES AVERAGED
a. Litter 3985 had no male fetuses.
** Significantly different from the control group value (p≤0.01).

Offspring (Malformations, Variations, etc.)

rAvPAL-PEG at 2 mg/kg/day significantly increased the percentage of fetuses per litter with any alterations (23.8 – 27.9% vs. 4.4% in controls) during the treatment periods of GD 11-16 and 15-20, but not with the treatment during GD 7-12.

However, treatment with 2 mg/kg/day increased the percentage of litters with any alteration (57.9 - 63% vs. 35% in controls) and fetuses with any alterations (13.3-29.6%% vs. 4.5% in controls) during all three treatment periods.

rAvPAL-PEG at 5 mg/kg/day produced significant increases in litters with any alteration (100% vs. 35% in controls), fetuses with any alterations (85.6-100% vs. 4.5% in controls) and percentage of fetuses per litter with any alterations (87.7 – 100% vs. 4.4% in controls) during the three treatment periods (GDs 7-12, 11-16 and 15-20).

As shown in the tables below (taken from the study report), rAvPAL-PEG at 5 mg/kg/ day significantly increased the number of litters and number of fetuses with external malformations of the head (e.g. eyes, ears, and jaw), body, and limbs during the three treatment periods. The incidence of external malformations in litters and fetuses are summarized in the tables below (taken from study report).

TABLE 9 (PAGE 2): FETAL GROSS EXTERNAL ALTERATIONS - CAESAREAN-DELIVERED LIVE FETUSES (DAY 29 OF GESTATION) - SUMMARY

GROUP		2	5
TEST MATERIALS		rAvPAL-PEG/ CONTROL ARTICLE	rAvPAL-PEG/ CONTROL ARTICLE
DOSE LEVELS (MG/KG)		2/0	5/0
DOSING (DAYS OF PRESUMED GESTATION)		7-12/13-20	7-12/13-20
LITTERS EVALUATED	N	19	19
LITTERS WITH LIVE FETUS(ES)	N	19	19
FETUSES EVALUATED	N	173	154
LIVE	N	173	153
DEAD a	N	0	1
EYE: LID(S) OPEN (M)			
LITTER INCIDENCE	N(%)	0(0.0)	14(73.7)**
FETAL INCIDENCE	N(%)	0(0.0)	86(56.2)**
EARS: ROTATED (M)			
LITTER INCIDENCE	N(%)	1(5.3)	14(73.7)**
FETAL INCIDENCE	N(%)	1(0.6)	72(47.0)**
JAW: MICROGNATHIA (M)			
LITTER INCIDENCE	N(%)	0(0.0)	8(42.1)**
FETAL INCIDENCE	N(%)	0(0.0)	38(24.8)**
FORE AND/OR HINDLIMBS: DIGIT(S) FUSED (M)			
LITTER INCIDENCE	N(%)	0(0.0)	12(63.2)**
FETAL INCIDENCE	N(%)	0(0.0)	54(35.3)**
FORE AND/OR HINDLIMBS: DIGIT(S) CONSTRICTED (M)			
LITTER INCIDENCE	N(%)	0(0.0)	4(21.0)**
FETAL INCIDENCE	N(%)	0(0.0)	4(2.6)**
PALATE: CLEFT (M)			
LITTER INCIDENCE	N(%)	0(0.0)	17(89.5)**
FETAL INCIDENCE	N(%)	0(0.0)	54(35.3)**
FORE AND/OR HINDLIMBS: ROTATED (M)			
LITTER INCIDENCE	N(%)	0(0.0)	13(68.4)**
FETAL INCIDENCE	N(%)	0(0.0)	74(48.4)**
FORE AND/OR HINDLIMBS: FLEXED (M)			
LITTER INCIDENCE	N(%)	0(0.0)	12(63.2)**
FETAL INCIDENCE	N(%)	0(0.0)	24(15.7)**

(M) = MALFORMATION
a. Dead fetus was excluded from summarization and statistical analyses. Observations for this conceptus is cited on Appendix 11.
** Significantly different from the control group value ($p \leq 0.01$).

TABLE 9 (PAGE 3): FETAL GROSS EXTERNAL ALTERATIONS - CAESAREAN-DELIVERED LIVE FETUSES (DAY 29 OF GESTATION) - SUMMARY

GROUP		2	5
TEST MATERIALS		rAvPAL-PEG/ CONTROL ARTICLE	rAvPAL-PEG/ CONTROL ARTICLE
DOSE LEVELS (MG/KG)		2/0	5/0
DOSING (DAYS OF PRESUMED GESTATION)		7-12/13-20	7-12/13-20
LITTERS EVALUATED	N	19	19
LITTERS WITH LIVE FETUS(ES)	N	19	19
FETUSES EVALUATED	N	173	154
LIVE	N	173	153
DEAD a	N	0	1
FORE AND/OR HINDLIMBS: DIGIT(S) ABSENT (M)			
LITTER INCIDENCE	N(%)	0(0.0)	12(63.2)**
FETAL INCIDENCE	N(%)	0(0.0)	27(17.6)**
FORE AND/OR HINDLIMBS: SHORT (M)			
LITTER INCIDENCE	N(%)	0(0.0)	6(31.6)**
FETAL INCIDENCE	N(%)	0(0.0)	25(16.3)**
HEAD: DOMED (M)			
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)
EYE: BULGE DEPRESSED (M)			
LITTER INCIDENCE	N(%)	0(0.0)	6(31.6)**
FETAL INCIDENCE	N(%)	0(0.0)	11(7.2)**
BODY: ABDOMINAL DISTENTION (M)			
LITTER INCIDENCE	N(%)	1(5.3)	3(15.8)
FETAL INCIDENCE	N(%)	1(0.6)	3(2.0)
BODY: ACCENTUATED FAT PADS (M)			
LITTER INCIDENCE	N(%)	0(0.0)	4(21.0)**
FETAL INCIDENCE	N(%)	0(0.0)	9(5.9)**
SNOUT: SHORT (M)			
LITTER INCIDENCE	N(%)	0(0.0)	8(42.1)**
FETAL INCIDENCE	N(%)	0(0.0)	37(24.2)**
FORE AND/OR HINDLIMBS: DIGIT(S) SPLAYED (M)			
LITTER INCIDENCE	N(%)	0(0.0)	6(31.6)**
FETAL INCIDENCE	N(%)	0(0.0)	7(4.6)**

(M) = MALFORMATION
a. Dead fetus was excluded from summarization and statistical analyses. Observations for this conceptus is cited on Appendix 11.
** Significantly different from the control group value (p≤0.01).

TABLE 9 (PAGE 4): FETAL GROSS EXTERNAL ALTERATIONS - CAESAREAN-DELIVERED LIVE FETUSES (DAY 29 OF GESTATION) - SUMMARY

GROUP		2	5
TEST MATERIALS		rAvPAL-PEG/ CONTROL ARTICLE	rAvPAL-PEG/ CONTROL ARTICLE
DOSE LEVELS (MG/KG)		2/0	5/0
DOSING (DAYS OF PRESUMED GESTATION)		7-12/13-20	7-12/13-20
LITTERS EVALUATED	N	19	19
LITTERS WITH LIVE FETUS(ES)	N	19	19
FETUSES EVALUATED	N	173	154
LIVE	N	173	153
DEAD a	N	0	1
TONGUE: PROTRUDES (M)			
LITTER INCIDENCE	N(%)	0(0.0)	5(26.3)**
FETAL INCIDENCE	N(%)	0(0.0)	10(6.5)**
EARS: LOW SET (M)			
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)
FETAL INCIDENCE	N(%)	0(0.0)	5(3.3)**
HEAD: MENINGOCELE (M)			
LITTER INCIDENCE	N(%)	0(0.0)	2(10.5)
FETAL INCIDENCE	N(%)	0(0.0)	2(1.3)
FORE AND/OR HINDLIMBS: DIGITS SHORT (M)			
LITTER INCIDENCE	N(%)	0(0.0)	5(26.3)**
FETAL INCIDENCE	N(%)	0(0.0)	8(5.2)**
BODY: UMBILICAL HERNIA (M)			
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)
BODY: EDEMA (M)			
LITTER INCIDENCE	N(%)	0(0.0)	5(26.3)**
FETAL INCIDENCE	N(%)	0(0.0)	6(3.9)**
BODY: THIN SKIN (M)			
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)
FETAL INCIDENCE	N(%)	0(0.0)	2(1.3)
HEAD: FIRM RAISED AREA			
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)
FETAL INCIDENCE	N(%)	0(0.0)	3(2.0)**

(M) = MALFORMATION
a. Dead fetus was excluded from summarization and statistical analyses. Observations for this conceptus is cited on Appendix 11.
** Significantly different from the control group value ($p \leq 0.01$).

TABLE 9 (PAGE 5): FETAL GROSS EXTERNAL ALTERATIONS - CAESAREAN-DELIVERED LIVE FETUSES (DAY 29 OF GESTATION) - SUMMARY

GROUP		3	6
TEST MATERIALS		CONTROL ARTICLE/ rAvPAL-PEG	CONTROL ARTICLE/ rAvPAL-PEG
DOSE LEVELS (MG/KG)		0/2	0/5
DOSING (DAYS OF PRESUMED GESTATION)		7-10,17-20/11-16	7-10,17-20/11-16
LITTERS EVALUATED	N	19	19
LITTERS WITH LIVE FETUS(ES)	N	19	19
FETUSES EVALUATED	N	162	160
LIVE	N	162	159
DEAD a	N	0	1
EYE: LID(S) OPEN (M)			
LITTER INCIDENCE	N(%)	3(15.8)	19(100.0)**
FETAL INCIDENCE	N(%)	6(3.7)	147(92.4)**
SNOUT: SHORT (M)			
LITTER INCIDENCE	N(%)	0(0.0)	12(63.2)**
FETAL INCIDENCE	N(%)	0(0.0)	65(40.9)**
PALATE: CLEFT (M)			
LITTER INCIDENCE	N(%)	2(10.5)	18(94.7)**
FETAL INCIDENCE	N(%)	6(3.7)	117(73.6)**
BODY: THIN SKIN (M)			
LITTER INCIDENCE	N(%)	0(0.0)	6(31.6)**
FETAL INCIDENCE	N(%)	0(0.0)	13(8.2)**
HEAD: DOMED (M)			
LITTER INCIDENCE	N(%)	0(0.0)	5(26.3)**
FETAL INCIDENCE	N(%)	0(0.0)	13(8.2)**
EARS: ROTATED (M)			
LITTER INCIDENCE	N(%)	1(5.3)	13(68.4)**
FETAL INCIDENCE	N(%)	1(0.6)	42(26.4)**
HEAD: MENINGOCELE (M)			
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)

(M) = MALFORMATION
a. Dead fetus was excluded from summarization and statistical analyses. Observations for this conceptus is cited on Appendix 11.
** Significantly different from the control group value ($p \leq 0.01$).

TABLE 9 (PAGE 6): FETAL GROSS EXTERNAL ALTERATIONS - CAESAREAN-DELIVERED LIVE FETUSES (DAY 29 OF GESTATION) - SUMMARY

GROUP		3	6
TEST MATERIALS		CONTROL ARTICLE/ rAvPAL-PEG	CONTROL ARTICLE/ rAvPAL-PEG
DOSE LEVELS (MG/KG)		0/2	0/5
DOSING (DAYS OF PRESUMED GESTATION)		7-10,17-20/11-16	7-10,17-20/11-16
LITTERS EVALUATED	N	19	19
LITTERS WITH LIVE FETUS(ES)	N	19	19
FETUSES EVALUATED	N	162	160
LIVE	N	162	159
DEAD a	N	0	1
BODY: EDEMA (M)			
LITTER INCIDENCE	N(%)	0(0.0)	10(52.6)**
FETAL INCIDENCE	N(%)	0(0.0)	21(13.2)**
FORE AND/OR HINDLIMBS: ROTATED (M)			
LITTER INCIDENCE	N(%)	0(0.0)	9(47.4)**
FETAL INCIDENCE	N(%)	0(0.0)	33(20.8)**
FORE AND/OR HINDLIMBS: FLEXED (M)			
LITTER INCIDENCE	N(%)	0(0.0)	2(10.5)
FETAL INCIDENCE	N(%)	0(0.0)	2(1.2)
JAW: MICROGNATHIA (M)			
LITTER INCIDENCE	N(%)	0(0.0)	11(57.9)**
FETAL INCIDENCE	N(%)	0(0.0)	59(37.1)**
FORE AND/OR HINDLIMBS: SHORT (M)			
LITTER INCIDENCE	N(%)	0(0.0)	8(42.1)**
FETAL INCIDENCE	N(%)	0(0.0)	16(10.1)**
TONGUE: PROTRUDES (M)			
LITTER INCIDENCE	N(%)	0(0.0)	10(52.6)**
FETAL INCIDENCE	N(%)	0(0.0)	55(34.6)**
HEAD: FIRM RAISED AREA (M)			
LITTER INCIDENCE	N(%)	0(0.0)	8(42.1)**
FETAL INCIDENCE	N(%)	0(0.0)	41(25.8)**
BODY: ABDOMINAL DISTENTION (M)			
LITTER INCIDENCE	N(%)	1(5.3)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)	0(0.0)

(M) = MALFORMATION
a. Dead fetus was excluded from summarization and statistical analyses. Observations for this conceptus is cited on Appendix 11.
** Significantly different from the control group value (p≤0.01).

TABLE 9 (PAGE 7): FETAL GROSS EXTERNAL ALTERATIONS - CAESAREAN-DELIVERED LIVE FETUSES (DAY 29 OF GESTATION) - SUMMARY

GROUP		3	6
TEST MATERIALS		CONTROL ARTICLE/ rAvPAL-PEG	CONTROL ARTICLE/ rAvPAL-PEG
DOSE LEVELS (MG/KG)		0/2	0/5
DOSING (DAYS OF PRESUMED GESTATION)		7-10,17-20/11-16	7-10,17-20/11-16
LITTERS EVALUATED	N	19	19
LITTERS WITH LIVE FETUS(ES)	N	19	19
FETUSES EVALUATED	N	162	160
LIVE	N	162	159
DEAD a	N	0	1
FORE AND/OR HINDLIMBS: DIGIT(S) SPLAYED (M)			
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)
EYE: BULGE DEPRESSED (M)			
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)
FETAL INCIDENCE	N(%)	0(0.0)	2(1.2)

(M) = MALFORMATION
a. Dead fetus was excluded from summarization and statistical analyses. Observations for this conceptus is cited on Appendix 11.

TABLE 9 (PAGE 8): FETAL GROSS EXTERNAL ALTERATIONS - CAESAREAN-DELIVERED LIVE FETUSES (DAY 29 OF GESTATION) - SUMMARY

GROUP		4	7
TEST MATERIALS		CONTROL ARTICLE/ rAvPAL-PEG	CONTROL ARTICLE/ rAvPAL-PEG
DOSE LEVELS (MG/KG)		0/2	0/5
DOSING (DAYS OF PRESUMED GESTATION)		7-14/15-20	7-14/15-20
LITTERS EVALUATED	N	20	15
LITTERS WITH LIVE FETUS(ES)	N	20	15
FETUSES EVALUATED	N	163	139
LIVE	N	163	139
FORE AND/OR HINDLIMBS: ROTATED (M)			
LITTER INCIDENCE	N(%)	1(5.0)	12(80.0)**
FETAL INCIDENCE	N(%)	1(0.6)	43(30.9)**
JAW: MICROGNATHIA (M)			
LITTER INCIDENCE	N(%)	1(5.0)	6(40.0)**
FETAL INCIDENCE	N(%)	1(0.6)	21(15.1)**
TONGUE: PROTRUDES (M)			
LITTER INCIDENCE	N(%)	1(5.0)	6(40.0)**
FETAL INCIDENCE	N(%)	1(0.6)	21(15.1)**
FORE AND/OR HINDLIMBS: SHORT (M)			
LITTER INCIDENCE	N(%)	0(0.0)	2(13.3)
FETAL INCIDENCE	N(%)	0(0.0)	7(5.0)**
BODY: EDEMA (M)			
LITTER INCIDENCE	N(%)	0(0.0)	13(86.7)**
FETAL INCIDENCE	N(%)	0(0.0)	102(73.4)**
HEAD: DOMED (M)			
LITTER INCIDENCE	N(%)	0(0.0)	11(73.3)**
FETAL INCIDENCE	N(%)	0(0.0)	76(54.7)**
EARS: ROTATED (M)			
LITTER INCIDENCE	N(%)	0(0.0)	12(80.0)**
FETAL INCIDENCE	N(%)	0(0.0)	68(48.9)**
EYE: LID(S) OPEN (M)			
LITTER INCIDENCE	N(%)	0(0.0)	6(40.0)**
FETAL INCIDENCE	N(%)	0(0.0)	11(7.9)**

(M) = MALFORMATION
** Significantly different from the control group value ($p \leq 0.01$).

TABLE 9 (PAGE 9): FETAL GROSS EXTERNAL ALTERATIONS - CAESAREAN-DELIVERED LIVE FETUSES (DAY 29 OF GESTATION) - SUMMARY

GROUP		4	7
TEST MATERIALS		CONTROL ARTICLE/ rAvPAL-PEG	CONTROL ARTICLE/ rAvPAL-PEG
DOSE LEVELS (MG/KG)		0/2	0/5
DOSING (DAYS OF PRESUMED GESTATION)		7-14/15-20	7-14/15-20
LITTERS EVALUATED	N	20	15
LITTERS WITH LIVE FETUS(ES)	N	20	15
FETUSES EVALUATED	N	163	139
LIVE	N	163	139
BODY: ABDOMINAL DISTENTION (M)			
LITTER INCIDENCE	N(%)	1(5.0)	2(13.3)
FETAL INCIDENCE	N(%)	1(0.6)	2(1.4)
BODY: THIN SKIN (M)			
LITTER INCIDENCE	N(%)	0(0.0)	1(6.7)
FETAL INCIDENCE	N(%)	0(0.0)	6(4.3)**
EYE: BULGE DEPRESSED (M)			
LITTER INCIDENCE	N(%)	0(0.0)	5(33.3)**
FETAL INCIDENCE	N(%)	0(0.0)	12(8.6)**
PALATE: CLEFT (M)			
LITTER INCIDENCE	N(%)	0(0.0)	1(6.7)
FETAL INCIDENCE	N(%)	0(0.0)	3(2.2)**

(M) = MALFORMATION
** Significantly different from the control group value (p≤0.01).

rAvPAL-PEG at 5 mg/kg/day significantly increased the incidence of soft tissue malformations in litters and fetuses, and the majority of malformations were observed in litters and fetuses from does treated during GD 7 to 12, as shown in the table below. The soft tissue malformations included discolored eyes, small eyes, corneal opacity (only with treatment during GD 15-20), small lungs, diaphragm hernia, and kidney abnormalities (small size, absent, or low set). No soft tissue malformations were observed in the group treated with 5 mg/kg/day during GD 11 to16.

Table 69: Treatment-Related Fetal Soft Tissue Malformations in Live fetuses (C-section Delivered on GDs 29) from the 5 mg/kg/day groups

Parameters		Dose groups (mg/kg/day)		
		5		
		Dosing days		
		7-12	11-16	15-20
EYES: DISCOLORED (M) LITTER INCIDENCE FETAL INCIDENCE	N (%)	12 (63.2)** 31(20.3)**	-	-
EYES: SMALL (M) LITTER INCIDENCE	N (%)	9 (47.4)** 20 (13.1)**	-	-

FETAL INCIDENCE				
EYES: CORNEAL OPACITY (M) LITTER INCIDENCE FETAL INCIDENCE	N (%)	-	-	14 (93.3)** 110 (79.1)**
LUNGS: SMALL (M) LITTER INCIDENCE FETAL INCIDENCE	N (%)	3 (15.8)** 3 (2.0)**	-	-
DIAPHRAGM: HERNIA (M) LITTER INCIDENCE FETAL INCIDENCE	N (%)	4 (21.0)** 7 (4.6)**	-	-
KIDNEYS: LOW SET (M) LITTER INCIDENCE FETAL INCIDENCE	N (%)	7 (36.8)** 8 (5.2)**	-	-
KIDNEYS: SMALL (M) LITTER INCIDENCE FETAL INCIDENCE	N (%)	14(73.7)** 50(32.7)**	-	-
KIDNEYS: ABSENT (M) LITTER INCIDENCE FETAL INCIDENCE	N (%)	6 (31.6)** 6 (3.9)**	-	-

-: Not found.
(M): malformation
** Significantly different from the control group value (p≤0.01).

Administration of 5 mg/kg/day produced significant increases in multiple types of malformations and variations in all regions of the skeletal system. Significant increases in malformations were observed in skull (e.g. incomplete ossification, anterior fontanelle irregularly shaped or large, interparietals or supraoccipitals not ossified, mandible incompletely ossified or shortened, maxillae shortened, palate and premaxilla incompletely ossified, and nasal bone shortened), sacral vertebrate (arches fused), caudal vertebrate (12th present), cervical vertebrae (centra fused), thoracic vertebrae

(arches fused), manubrium of sternum (duplicated), sternal centra (duplicated), xiphoid (duplicated), claviculae (not ossified), limbs (e.g. short or small radius, or radius not ossified, shortened ulna/humerus/femur, absence of metacarpal/metatarsal, digits, and phalanx, fused metacarpals/metatarsals, digits and phalanges, bent radius/tibia/fibula/ humerus/femur, short fore/hind limb, shortened tibia and fibula, fused tibia and fibula, bowed fibula, hind limb not ossified, and malpositioned tibia and fibula), and pelvis (short ilium).

Increased incidence of skeletal variations was observed in skull, vertebrae, ribs, limbs, pelvis, claviculae, sternum, and scapulae in the 5 mg/kg/day groups. In addition, rAvPAL-PEG at 5 mg/kg/day significantly reduced the fetal ossification sites in hyoid bones, caudal vertebrae, sternal centers, xiphoid, forelimb metacarpals, forelimb phalanges, hindlimb tarsals, and hindlimb phalanges.

The incidence of skeletal malformations and variations in litters and fetuses in the control, 2, and 5 mg/kg/day groups are summarized in the tables below (taken from the study report).

TABLE 11 (PAGE 1): FETAL SKELETAL ALTERATIONS - CAESAREAN-DELIVERED LIVE FETUSES (DAY 29 OF GESTATION) - SUMMARY
(See footnotes on the last page of this table.)

GROUP		1
TEST MATERIALS		CONTROL ARTICLE
DOSE LEVELS (MG/KG)		0
DOSING (DAYS OF PRESUMED GESTATION)		7-20
LITTERS EVALUATED	N	20
LITTERS WITH LIVE FETUS(ES)	N	20
FETUSES EVALUATED a	N	177
LIVE	N	177
SKULL: NASAL - FRONTAL, SUTURE IRREGULAR (V)		
LITTER INCIDENCE	N(%)	1(5.0)
FETAL INCIDENCE	N(%)	1(0.6)
HYOID: ALA, ANGULATED (V)		
LITTER INCIDENCE	N(%)	5(25.0)
FETAL INCIDENCE	N(%)	5(2.8)
CAUDAL VERTEBRAE: MISALIGNED (V)		
LITTER INCIDENCE	N(%)	1(5.0)
FETAL INCIDENCE	N(%)	1(0.6)
MANUBRIUM: IRREGULARLY SHAPED (V)		
LITTER INCIDENCE	N(%)	1(5.0)
FETAL INCIDENCE	N(%)	1(0.6)
STERNAL CENTRA: FUSED (V)		
LITTER INCIDENCE	N(%)	3(15.0)
FETAL INCIDENCE	N(%)	3(1.7)
STERNAL CENTRA: ASYMMETRIC (V)		
LITTER INCIDENCE	N(%)	1(5.0)
FETAL INCIDENCE	N(%)	1(0.6)
XIPHOID: DUPLICATED (M)		
LITTER INCIDENCE	N(%)	1(5.0)
FETAL INCIDENCE	N(%)	1(0.6)

(M) = MALFORMATION (V) = VARIATION

TABLE 11 (PAGE 2): FETAL SKELETAL ALTERATIONS - CAESAREAN-DELIVERED LIVE FETUSES (DAY 29 OF GESTATION) - SUMMARY
(See footnotes on the last page of this table.)

GROUP		2	5
TEST MATERIALS		rAvPAL-PEG/ CONTROL ARTICLE	rAvPAL-PEG/ CONTROL ARTICLE
DOSE LEVELS (MG/KG)		2/0	5/0
DOSING (DAYS OF PRESUMED GESTATION)		7-12/13-20	7-12/13-20
LITTERS EVALUATED	N	19	19
LITTERS WITH LIVE FETUS(ES)	N	19	19
FETUSES EVALUATED a	N	173	154
LIVE	N	173	153
DEAD b	N	0	1
SKULL: FRONTALS, CONTAIN AN INTERFRONTAL (V)			
LITTER INCIDENCE	N(%)	1(5.3)	4(21.0)
FETAL INCIDENCE	N(%)	1(0.6)	9(5.9)**
SKULL: PALATE, INCOMPLETELY OSSIFIED			
LITTER INCIDENCE	N(%)	0(0.0)	15(78.9)**
FETAL INCIDENCE	N(%)	0(0.0)	40(26.1)**
SKULL: NASALS, CONTAIN AN INTERNASAL (V)			
LITTER INCIDENCE	N(%)	0(0.0)	2(10.5)
FETAL INCIDENCE	N(%)	0(0.0)	4(2.6)**
SKULL: EYE SOCKET, SMALL (M)			
LITTER INCIDENCE	N(%)	0(0.0)	7(36.8)**
FETAL INCIDENCE	N(%)	0(0.0)	13(8.5)**
SKULL: NASALS, MIDLINE SUTURE DISPLACED (V)			
LITTER INCIDENCE	N(%)	2(10.5)	3(15.8)
FETAL INCIDENCE	N(%)	2(1.2)	5(3.3)**
SKULL: INTERPARIETALS, INCOMPLETELY OSSIFIED (V)			
LITTER INCIDENCE	N(%)	0(0.0)	11(57.9)**
FETAL INCIDENCE	N(%)	0(0.0)	33(21.6)**
SKULL: SUPRAOCCIPITALS, IRREGULARLY SHAPED (V)			
LITTER INCIDENCE	N(%)	0(0.0)	11(57.9)**
FETAL INCIDENCE	N(%)	0(0.0)	31(20.3)**
SKULL: ANTERIOR FONTANELLE, IRREGULARLY SHAPED (M)			
LITTER INCIDENCE	N(%)	0(0.0)	14(73.7)**
FETAL INCIDENCE	N(%)	0(0.0)	61(39.9)**

(M) = MALFORMATION (V) = VARIATION
** Significantly different from the control group value ($p \leq 0.01$).

TABLE 11 (PAGE 3): FETAL SKELETAL ALTERATIONS - CAESAREAN-DELIVERED LIVE FETUSES (DAY 29 OF GESTATION) - SUMMARY
(See footnotes on the last page of this table.)

GROUP		2	5
TEST MATERIALS		rAvPAL-PEG/ CONTROL ARTICLE	rAvPAL-PEG/ CONTROL ARTICLE
DOSE LEVELS (MG/KG)		2/0	5/0
DOSING (DAYS OF PRESUMED GESTATION)		7-12/13-20	7-12/13-20
LITTERS EVALUATED	N	19	19
LITTERS WITH LIVE FETUS(ES)	N	19	19
FETUSES EVALUATED a	N	173	154
LIVE	N	173	153
DEAD b	N	0	1
SKULL: INTERPARIETALS, NOT OSSIFIED (M)			
LITTER INCIDENCE	N(%)	0(0.0)	7(36.8)**
FETAL INCIDENCE	N(%)	0(0.0)	25(16.3)**
SKULL: FRONTALS, SUTURE IRREGULARLY SHAPED (V)			
LITTER INCIDENCE	N(%)	0(0.0)	3(15.8)**
FETAL INCIDENCE	N(%)	0(0.0)	7(4.6)**
SKULL: FRONTAL, CONTAINS AN INTRAFRONTAL (V)			
LITTER INCIDENCE	N(%)	0(0.0)	6(31.6)**
FETAL INCIDENCE	N(%)	0(0.0)	6(3.9)**
SKULL: NASAL, IRREGULARLY SHAPED (V)			
LITTER INCIDENCE	N(%)	0(0.0)	2(10.5)
FETAL INCIDENCE	N(%)	0(0.0)	2(1.3)
SKULL: NASAL, INCOMPLETELY OSSIFIED (V)			
LITTER INCIDENCE	N(%)	0(0.0)	8(42.1)**
FETAL INCIDENCE	N(%)	0(0.0)	30(19.6)**
SKULL: FRONTALS, SUTURE LARGE (V)			
LITTER INCIDENCE	N(%)	0(0.0)	3(15.8)**
FETAL INCIDENCE	N(%)	0(0.0)	4(2.6)**
SKULL: FRONTAL, INCOMPLETELY OSSIFIED (V)			
LITTER INCIDENCE	N(%)	0(0.0)	9(47.4)**
FETAL INCIDENCE	N(%)	0(0.0)	33(21.6)**

(M) = MALFORMATION (V) = VARIATION
** Significantly different from the control group value ($p \leq 0.01$).

TABLE 11 (PAGE 4): FETAL SKELETAL ALTERATIONS - CAESAREAN-DELIVERED LIVE FETUSES (DAY 29 OF GESTATION) - SUMMARY
(See footnotes on the last page of this table.)

GROUP		2	5
TEST MATERIALS		rAvPAL-PEG/ CONTROL ARTICLE	rAvPAL-PEG/ CONTROL ARTICLE
DOSE LEVELS (MG/KG)		2/0	5/0
DOSING (DAYS OF PRESUMED GESTATION)		7-12/13-20	7-12/13-20
LITTERS EVALUATED	N	19	19
LITTERS WITH LIVE FETUS(ES)	N	19	19
FETUSES EVALUATED a	N	173	154
LIVE	N	173	153
DEAD b	N	0	1
SKULL: NASAL, CONTAINS AN INTRANASAL (V)			
LITTER INCIDENCE	N(%)	0(0.0)	5(26.3)**
FETAL INCIDENCE	N(%)	0(0.0)	6(3.9)**
SKULL: FRONTALS, SUTURE IRREGULAR (V)			
LITTER INCIDENCE	N(%)	0(0.0)	2(10.5)
FETAL INCIDENCE	N(%)	0(0.0)	4(2.6)**
SKULL: SUPRAOCCIPITALS, NOT OSSIFIED (M)			
LITTER INCIDENCE	N(%)	0(0.0)	2(10.5)
FETAL INCIDENCE	N(%)	0(0.0)	8(5.2)**
SKULL: TYMPANIC RING, SMALL (M)			
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)
SKULL: MANDIBLE, INCOMPLETELY OSSIFIED (M)			
LITTER INCIDENCE	N(%)	0(0.0)	2(10.5)
FETAL INCIDENCE	N(%)	0(0.0)	2(1.3)
SKULL: PREMAXILLA, NOT OSSIFIED (M)			
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)
SKULL: MANDIBLES, SHORT (M)			
LITTER INCIDENCE	N(%)	0(0.0)	7(36.8)**
FETAL INCIDENCE	N(%)	0(0.0)	21(13.7)**
SKULL: MAXILLAE, SHORT (M)			
LITTER INCIDENCE	N(%)	0(0.0)	7(36.8)**
FETAL INCIDENCE	N(%)	0(0.0)	24(15.7)**

(M) = MALFORMATION (V) = VARIATION
** Significantly different from the control group value ($p \leq 0.01$).

TABLE 11 (PAGE 5): FETAL SKELETAL ALTERATIONS - CAESAREAN-DELIVERED LIVE FETUSES (DAY 29 OF GESTATION) - SUMMARY
(See footnotes on the last page of this table.)

GROUP		2	5
TEST MATERIALS		rAvPAL-PEG/ CONTROL ARTICLE	rAvPAL-PEG/ CONTROL ARTICLE
DOSE LEVELS (MG/KG)		2/0	5/0
DOSING (DAYS OF PRESUMED GESTATION)		7-12/13-20	7-12/13-20
LITTERS EVALUATED	N	19	19
LITTERS WITH LIVE FETUS(ES)	N	19	19
FETUSES EVALUATED a	N	173	154
LIVE	N	173	153
DEAD b	N	0	1
SKULL: INCOMPLETELY OSSIFIED (M)			
LITTER INCIDENCE	N(%)	0(0.0)	6(31.6)**
FETAL INCIDENCE	N(%)	0(0.0)	32(20.9)**
SKULL: ANTERIOR FONTANELLE, LARGE (M)			
LITTER INCIDENCE	N(%)	0(0.0)	2(10.5)
FETAL INCIDENCE	N(%)	0(0.0)	2(1.3)
SKULL: NOT OSSIFIED (M)			
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)
FETAL INCIDENCE	N(%)	0(0.0)	3(2.0)**
SKULL: PARIETAL, INCOMPLETELY OSSIFIED (V)			
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)
SKULL: FUSED (M)			
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)
HYOID: ALA, ANGULATED (V)			
LITTER INCIDENCE	N(%)	8(42.1)	13(68.4)**
FETAL INCIDENCE	N(%)	12(6.9)	34(22.2)**
HYOID: ALA, INCOMPLETELY OSSIFIED (V)			
LITTER INCIDENCE	N(%)	0(0.0)	3(15.8)**
FETAL INCIDENCE	N(%)	0(0.0)	3(2.0)**
HYOID: ALA, NOT OSSIFIED (V)			
LITTER INCIDENCE	N(%)	0(0.0)	4(21.0)**
FETAL INCIDENCE	N(%)	0(0.0)	7(4.6)**

(M) = MALFORMATION (V) = VARIATION
** Significantly different from the control group value ($p \leq 0.01$).

TABLE 11 (PAGE 6): FETAL SKELETAL ALTERATIONS - CAESAREAN-DELIVERED LIVE FETUSES (DAY 29 OF GESTATION) - SUMMARY
(See footnotes on the last page of this table.)

GROUP		2	5
TEST MATERIALS		rAvPAL-PEG/ CONTROL ARTICLE	rAvPAL-PEG/ CONTROL ARTICLE
DOSE LEVELS (MG/KG)		2/0	5/0
DOSING (DAYS OF PRESUMED GESTATION)		7-12/13-20	7-12/13-20
LITTERS EVALUATED	N	19	19
LITTERS WITH LIVE FETUS(ES)	N	19	19
FETUSES EVALUATED a	N	173	154
LIVE	N	173	153
DEAD b	N	0	1
HYOID: ALA, SHORT (V)			
LITTER INCIDENCE	N(%)	0(0.0)	6(31.6)**
FETAL INCIDENCE	N(%)	0(0.0)	7(4.6)**
HYOID: ALA, SMALL (V)			
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)
CERVICAL VERTEBRAE: CENTRUM, UNILATERAL OSSIFICATION (V)			
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)
THORACIC VERTEBRAE: ARCHES, FUSED (M)			
LITTER INCIDENCE	N(%)	0(0.0)	4(21.0)**
FETAL INCIDENCE	N(%)	0(0.0)	10(6.5)**
LUMBAR VERTEBRAE: ARCHES, CLOSE SET (V)			
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)
LUMBAR VERTEBRAE: ARCH, IRREGULARLY SHAPED (V)			
LITTER INCIDENCE	N(%)	0(0.0)	2(10.5)
FETAL INCIDENCE	N(%)	0(0.0)	2(1.3)
LUMBAR VERTEBRAE: ARCHES, FUSED (M)			
LITTER INCIDENCE	N(%)	0(0.0)	2(10.5)
FETAL INCIDENCE	N(%)	0(0.0)	8(5.2)**
SACRAL VERTEBRAE: ARCH, IRREGULARLY SHAPED (V)			
LITTER INCIDENCE	N(%)	0(0.0)	2(10.5)
FETAL INCIDENCE	N(%)	0(0.0)	2(1.3)

(M) = MALFORMATION (V) = VARIATION
** Significantly different from the control group value ($p \leq 0.01$).

TABLE 11 (PAGE 7): FETAL SKELETAL ALTERATIONS - CAESAREAN-DELIVERED LIVE FETUSES (DAY 29 OF GESTATION) - SUMMARY
(See footnotes on the last page of this table.)

GROUP		2	5
TEST MATERIALS		rAvPAL-PEG/ CONTROL ARTICLE	rAvPAL-PEG/ CONTROL ARTICLE
DOSE LEVELS (MG/KG)		2/0	5/0
DOSING (DAYS OF PRESUMED GESTATION)		7-12/13-20	7-12/13-20
LITTERS EVALUATED	N	19	19
LITTERS WITH LIVE FETUS(ES)	N	19	19
FETUSES EVALUATED a	N	173	154
LIVE	N	173	153
DEAD b	N	0	1
SACRAL VERTEBRAE: ARCHES, FUSED (M)			
LITTER INCIDENCE	N(%)	0(0.0)	10(52.6)**
FETAL INCIDENCE	N(%)	0(0.0)	29(19.0)**
SACRAL VERTEBRAE: ARCH, SMALL (V)			
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)
CAUDAL VERTEBRAE: SPACE (M)			
LITTER INCIDENCE	N(%)	0(0.0)	3(15.8)**
FETAL INCIDENCE	N(%)	0(0.0)	3(2.0)**
CAUDAL VERTEBRAE: MISALIGNED (V)			
LITTER INCIDENCE	N(%)	0(0.0)	11(57.9)**
FETAL INCIDENCE	N(%)	0(0.0)	17(11.1)**
CAUDAL VERTEBRAE: FUSED (M)			
LITTER INCIDENCE	N(%)	0(0.0)	3(15.8)**
FETAL INCIDENCE	N(%)	0(0.0)	3(2.0)**
CAUDAL VERTEBRAE: ARCHES, CLOSE SET (V)			
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)
CAUDAL VERTEBRAE: INCOMPLETELY OSSIFIED (V)			
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)
CAUDAL VERTEBRAE: SMALL (V)			
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)

(M) = MALFORMATION (V) = VARIATION
** Significantly different from the control group value ($p \leq 0.01$).

TABLE 11 (PAGE 8): FETAL SKELETAL ALTERATIONS - CAESAREAN-DELIVERED LIVE FETUSES (DAY 29 OF GESTATION) - SUMMARY
(See footnotes on the last page of this table.)

GROUP		2	5
TEST MATERIALS		rAvPAL-PEG/ CONTROL ARTICLE	rAvPAL-PEG/ CONTROL ARTICLE
DOSE LEVELS (MG/KG)		2/0	5/0
DOSING (DAYS OF PRESUMED GESTATION)		7-12/13-20	7-12/13-20
LITTERS EVALUATED	N	19	19
LITTERS WITH LIVE FETUS(ES)	N	19	19
FETUSES EVALUATED a	N	173	154
LIVE	N	173	153
DEAD b	N	0	1
RIBS: FLAT (V)			
LITTER INCIDENCE	N(%)	1(5.3)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)	0(0.0)
RIBS: THICKENED (V)			
LITTER INCIDENCE	N(%)	1(5.3)	2(10.5)
FETAL INCIDENCE	N(%)	1(0.6)	2(1.3)
RIBS: BOWED (V)			
LITTER INCIDENCE	N(%)	0(0.0)	7(36.8)**
FETAL INCIDENCE	N(%)	0(0.0)	10(6.5)**
RIBS: SHORT (V)			
LITTER INCIDENCE	N(%)	0(0.0)	10(52.6)**
FETAL INCIDENCE	N(%)	0(0.0)	58(37.9)**
RIBS: FUSED (V)			
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)
RIBS: IRREGULARLY SHAPED (V)			
LITTER INCIDENCE	N(%)	0(0.0)	3(15.8)**
FETAL INCIDENCE	N(%)	0(0.0)	7(4.6)**
RIBS: INCOMPLETELY OSSIFIED (V)			
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)
MANUBRIUM: DUPLICATED (M)			
LITTER INCIDENCE	N(%)	0(0.0)	8(42.1)**
FETAL INCIDENCE	N(%)	0(0.0)	21(13.7)**

(M) = MALFORMATION (V) = VARIATION
** Significantly different from the control group value ($p \leq 0.01$).

TABLE 11 (PAGE 9): FETAL SKELETAL ALTERATIONS - CAESAREAN-DELIVERED LIVE FETUSES (DAY 29 OF GESTATION) - SUMMARY
(See footnotes on the last page of this table.)

GROUP		2	5
TEST MATERIALS		rAvPAL-PEG/ CONTROL ARTICLE	rAvPAL-PEG/ CONTROL ARTICLE
DOSE LEVELS (MG/KG)		2/0	5/0
DOSING (DAYS OF PRESUMED GESTATION)		7-12/13-20	7-12/13-20
LITTERS EVALUATED	N	19	19
LITTERS WITH LIVE FETUS(ES)	N	19	19
FETUSES EVALUATED a	N	173	154
LIVE	N	173	153
DEAD b	N	0	1
MANUBRIUM: LARGE (V)			
LITTER INCIDENCE	N(%)	0(0.0)	4(21.0)**
FETAL INCIDENCE	N(%)	0(0.0)	7(4.6)**
MANUBRIUM: IRREGULARLY SHAPED (V)			
LITTER INCIDENCE	N(%)	0(0.0)	12(63.2)**
FETAL INCIDENCE	N(%)	0(0.0)	26(17.0)**
MANUBRIUM: FUSED (V)			
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)
FETAL INCIDENCE	N(%)	0(0.0)	2(1.3)
STERNAL CENTRA: IRREGULARLY SHAPED (V)			
LITTER INCIDENCE	N(%)	0(0.0)	10(52.6)**
FETAL INCIDENCE	N(%)	0(0.0)	13(8.5)**
STERNAL CENTRA: FUSED (V)			
LITTER INCIDENCE	N(%)	3(15.8)	12(63.2)**
FETAL INCIDENCE	N(%)	7(4.0)	51(33.3)**
STERNAL CENTRA: DUPLICATED (M)			
LITTER INCIDENCE	N(%)	0(0.0)	8(42.1)**
FETAL INCIDENCE	N(%)	0(0.0)	18(11.8)**
STERNAL CENTRA: INCOMPLETELY OSSIFIED (V)			
LITTER INCIDENCE	N(%)	0(0.0)	8(42.1)**
FETAL INCIDENCE	N(%)	0(0.0)	15(9.8)**
STERNAL CENTRA: LARGE (V)			
LITTER INCIDENCE	N(%)	0(0.0)	5(26.3)**
FETAL INCIDENCE	N(%)	0(0.0)	11(7.2)**

(M) = MALFORMATION (V) = VARIATION
** Significantly different from the control group value ($p \leq 0.01$).

TABLE 11 (PAGE 10): FETAL SKELETAL ALTERATIONS - CAESAREAN-DELIVERED LIVE FETUSES (DAY 29 OF GESTATION) - SUMMARY
(See footnotes on the last page of this table.)

GROUP		2	5
TEST MATERIALS		rAvPAL-PEG/ CONTROL ARTICLE	rAvPAL-PEG/ CONTROL ARTICLE
DOSE LEVELS (MG/KG)		2/0	5/0
DOSING (DAYS OF PRESUMED GESTATION)		7-12/13-20	7-12/13-20
LITTERS EVALUATED	N	19	19
LITTERS WITH LIVE FETUS(ES)	N	19	19
FETUSES EVALUATED a	N	173	154
LIVE	N	173	153
DEAD b	N	0	1
STERNAL CENTRA: NOT OSSIFIED (V)			
LITTER INCIDENCE	N(%)	0(0.0)	5(26.3)**
FETAL INCIDENCE	N(%)	0(0.0)	6(3.9)**
STERNAL CENTRA: ASYMMETRIC (V)			
LITTER INCIDENCE	N(%)	0(0.0)	3(15.8)**
FETAL INCIDENCE	N(%)	0(0.0)	3(2.0)**
STERNAL CENTRA: MISALIGNED (V)			
LITTER INCIDENCE	N(%)	0(0.0)	2(10.5)
FETAL INCIDENCE	N(%)	0(0.0)	2(1.3)
XIPHOID: DUPLICATED (M)			
LITTER INCIDENCE	N(%)	0(0.0)	6(31.6)**
FETAL INCIDENCE	N(%)	0(0.0)	14(9.2)**
XIPHOID: LARGE (V)			
LITTER INCIDENCE	N(%)	0(0.0)	7(36.8)**
FETAL INCIDENCE	N(%)	0(0.0)	9(5.9)**
XIPHOID: IRREGULARLY SHAPED (V)			
LITTER INCIDENCE	N(%)	0(0.0)	12(63.2)**
FETAL INCIDENCE	N(%)	0(0.0)	29(19.0)**
XIPHOID: FUSED (V)			
LITTER INCIDENCE	N(%)	0(0.0)	8(42.1)**
FETAL INCIDENCE	N(%)	0(0.0)	27(17.6)**
XIPHOID: MISALIGNED (V)			
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)

(M) = MALFORMATION (V) = VARIATION
** Significantly different from the control group value ($p \leq 0.01$).

TABLE 11 (PAGE 11): FETAL SKELETAL ALTERATIONS - CAESAREAN-DELIVERED LIVE FETUSES (DAY 29 OF GESTATION) - SUMMARY
(See footnotes on the last page of this table.)

GROUP		2	5
TEST MATERIALS		rAvPAL-PEG/ CONTROL ARTICLE	rAvPAL-PEG/ CONTROL ARTICLE
DOSE LEVELS (MG/KG)		2/0	5/0
DOSING (DAYS OF PRESUMED GESTATION)		7-12/13-20	7-12/13-20
LITTERS EVALUATED	N	19	19
LITTERS WITH LIVE FETUS(ES)	N	19	19
FETUSES EVALUATED a	N	173	154
LIVE	N	173	153
DEAD b	N	0	1
XIPHOID: SMALL (V)			
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)
CLAVICULAE: SMALL (V)			
LITTER INCIDENCE	N(%)	0(0.0)	8(42.1)**
FETAL INCIDENCE	N(%)	0(0.0)	34(22.2)**
CLAVICULAE: INCOMPLETELY OSSIFIED (V)			
LITTER INCIDENCE	N(%)	0(0.0)	2(10.5)
FETAL INCIDENCE	N(%)	0(0.0)	6(3.9)**
CLAVICULAE: NOT OSSIFIED (M)			
LITTER INCIDENCE	N(%)	0(0.0)	5(26.3)**
FETAL INCIDENCE	N(%)	0(0.0)	8(5.2)**
SCAPULAE: ALA, MALPOSITIONED (V)			
LITTER INCIDENCE	N(%)	0(0.0)	14(73.7)**
FETAL INCIDENCE	N(%)	0(0.0)	81(52.9)**
SCAPULAE: BODY, THIN (V)			
LITTER INCIDENCE	N(%)	0(0.0)	14(73.7)**
FETAL INCIDENCE	N(%)	0(0.0)	80(52.3)**
SCAPULAE: ALA, IRREGULARLY SHAPED (V)			
LITTER INCIDENCE	N(%)	0(0.0)	3(15.8)**
FETAL INCIDENCE	N(%)	0(0.0)	3(2.0)**
SCAPULAE: ALA, SHORT (V)			
LITTER INCIDENCE	N(%)	0(0.0)	3(15.8)**
FETAL INCIDENCE	N(%)	0(0.0)	3(2.0)**

(M) = MALFORMATION (V) = VARIATION
** Significantly different from the control group value ($p \leq 0.01$).

TABLE 11 (PAGE 12): FETAL SKELETAL ALTERATIONS - CAESAREAN-DELIVERED LIVE FETUSES (DAY 29 OF GESTATION) - SUMMARY
(See footnotes on the last page of this table.)

GROUP		2	5
TEST MATERIALS		rAvPAL-PEG/ CONTROL ARTICLE	rAvPAL-PEG/ CONTROL ARTICLE
DOSE LEVELS (MG/KG)		2/0	5/0
DOSING (DAYS OF PRESUMED GESTATION)		7-12/13-20	7-12/13-20
LITTERS EVALUATED	N	19	19
LITTERS WITH LIVE FETUS(ES)	N	19	19
FETUSES EVALUATED a	N	173	154
LIVE	N	173	153
DEAD b	N	0	1
SCAPULAE: ALA, WAVY (V)			
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)
FETAL INCIDENCE	N(%)	0(0.0)	2(1.3)
SCAPULAE: BODY, IRREGULARLY SHAPED (V)			
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)
FORE LIMB: RADIUS, SHORT (M)			
LITTER INCIDENCE	N(%)	0(0.0)	8(42.1)**
FETAL INCIDENCE	N(%)	0(0.0)	34(22.2)**
FORE LIMB: ULNA, SHORT (M)			
LITTER INCIDENCE	N(%)	0(0.0)	7(36.8)**
FETAL INCIDENCE	N(%)	0(0.0)	30(19.6)**
FORE LIMB: METACARPAL, ABSENT (M)			
LITTER INCIDENCE	N(%)	0(0.0)	8(42.1)**
FETAL INCIDENCE	N(%)	0(0.0)	17(11.1)**
FORE LIMB: DIGIT, ABSENT (M)			
LITTER INCIDENCE	N(%)	0(0.0)	12(63.2)**
FETAL INCIDENCE	N(%)	0(0.0)	27(17.6)**
FORE LIMB: PHALANX, ABSENT (M)			
LITTER INCIDENCE	N(%)	0(0.0)	7(36.8)**
FETAL INCIDENCE	N(%)	0(0.0)	17(11.1)**
FORE LIMB: METACARPALS, FUSED (M)			
LITTER INCIDENCE	N(%)	0(0.0)	12(63.2)**
FETAL INCIDENCE	N(%)	0(0.0)	30(19.6)**

(M) = MALFORMATION (V) = VARIATION
** Significantly different from the control group value ($p \leq 0.01$).

TABLE 11 (PAGE 13): FETAL SKELETAL ALTERATIONS - CAESAREAN-DELIVERED LIVE FETUSES (DAY 29 OF GESTATION) - SUMMARY
(See footnotes on the last page of this table.)

GROUP		2	5
TEST MATERIALS		rAvPAL-PEG/ CONTROL ARTICLE	rAvPAL-PEG/ CONTROL ARTICLE
DOSE LEVELS (MG/KG)		2/0	5/0
DOSING (DAYS OF PRESUMED GESTATION)		7-12/13-20	7-12/13-20
LITTERS EVALUATED	N	19	19
LITTERS WITH LIVE FETUS(ES)	N	19	19
FETUSES EVALUATED a	N	173	154
LIVE	N	173	153
DEAD b	N	0	1
FORE LIMB: PHALANX, SMALL (V)			
LITTER INCIDENCE	N(%)	0(0.0)	4(21.0)**
FETAL INCIDENCE	N(%)	0(0.0)	5(3.3)**
FORE LIMB: PHALANGES, FUSED (M)			
LITTER INCIDENCE	N(%)	0(0.0)	7(36.8)**
FETAL INCIDENCE	N(%)	0(0.0)	18(11.8)**
FORE LIMB: RADIUS, BENT (M)			
LITTER INCIDENCE	N(%)	0(0.0)	4(21.0)**
FETAL INCIDENCE	N(%)	0(0.0)	9(5.9)**
FORE LIMB: RADIUS, NOT OSSIFIED (M)			
LITTER INCIDENCE	N(%)	0(0.0)	2(10.5)
FETAL INCIDENCE	N(%)	0(0.0)	4(2.6)**
FORE LIMB: DIGITS, FUSED (M)			
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)
FETAL INCIDENCE	N(%)	0(0.0)	2(1.3)
FORE LIMB: SHORT (M)			
LITTER INCIDENCE	N(%)	0(0.0)	6(31.6)**
FETAL INCIDENCE	N(%)	0(0.0)	33(21.6)**
FORE LIMB: RADIUS, SMALL (M)			
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)
PELVIS: PUBIS, NOT OSSIFIED (V)			
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)

(M) = MALFORMATION (V) = VARIATION
** Significantly different from the control group value ($p \leq 0.01$).

TABLE 11 (PAGE 14): FETAL SKELETAL ALTERATIONS - CAESAREAN-DELIVERED LIVE FETUSES (DAY 29 OF GESTATION) - SUMMARY
(See footnotes on the last page of this table.)

GROUP			2	5
TEST MATERIALS			rAvPAL-PEG/ CONTROL ARTICLE	rAvPAL-PEG/ CONTROL ARTICLE
DOSE LEVELS (MG/KG)			2/0	5/0
DOSING (DAYS OF PRESUMED GESTATION)			7-12/13-20	7-12/13-20
LITTERS EVALUATED		N	19	19
LITTERS WITH LIVE FETUS(ES)		N	19	19
FETUSES EVALUATED a		N	173	154
LIVE		N	173	153
DEAD b		N	0	1
PELVIS: ILIUM, SHORT (M)				
LITTER INCIDENCE		N(%)	0(0.0)	4(21.0)**
FETAL INCIDENCE		N(%)	0(0.0)	9(5.9)**
HIND LIMB: TIBIA, SHORT (M)				
LITTER INCIDENCE		N(%)	0(0.0)	6(31.6)**
FETAL INCIDENCE		N(%)	0(0.0)	14(9.2)**
HIND LIMB: FIBULA, SHORT (M)				
LITTER INCIDENCE		N(%)	0(0.0)	10(52.6)**
FETAL INCIDENCE		N(%)	0(0.0)	46(30.1)**
HIND LIMB: TIBIA AND FIBULA, FUSED (M)				
LITTER INCIDENCE		N(%)	0(0.0)	3(15.8)**
FETAL INCIDENCE		N(%)	0(0.0)	4(2.6)**
HIND LIMB: TIBIA, NOT OSSIFIED (M)				
LITTER INCIDENCE		N(%)	0(0.0)	12(63.2)**
FETAL INCIDENCE		N(%)	0(0.0)	52(34.0)**
HIND LIMB: FIBULA, BOWED (M)				
LITTER INCIDENCE		N(%)	0(0.0)	8(42.1)**
FETAL INCIDENCE		N(%)	0(0.0)	28(18.3)**
HIND LIMB: METATARSAL, ABSENT (M)				
LITTER INCIDENCE		N(%)	0(0.0)	4(21.0)**
FETAL INCIDENCE		N(%)	0(0.0)	6(3.9)**
HIND LIMB: DIGIT, ABSENT (M)				
LITTER INCIDENCE		N(%)	0(0.0)	2(10.5)
FETAL INCIDENCE		N(%)	0(0.0)	5(3.3)**

(M) = MALFORMATION (V) = VARIATION
** Significantly different from the control group value ($p \leq 0.01$).

TABLE 11 (PAGE 15): FETAL SKELETAL ALTERATIONS - CAESAREAN-DELIVERED LIVE FETUSES (DAY 29 OF GESTATION) - SUMMARY
(See footnotes on the last page of this table.)

GROUP		2	5
TEST MATERIALS		rAvPAL-PEG/ CONTROL ARTICLE	rAvPAL-PEG/ CONTROL ARTICLE
DOSE LEVELS (MG/KG)		2/0	5/0
DOSING (DAYS OF PRESUMED GESTATION)		7-12/13-20	7-12/13-20
LITTERS EVALUATED	N	19	19
LITTERS WITH LIVE FETUS(ES)	N	19	19
FETUSES EVALUATED a	N	173	154
LIVE	N	173	153
DEAD b	N	0	1
HIND LIMB: METATARSALS, FUSED (M)			
LITTER INCIDENCE	N(%)	0(0.0)	10(52.6)**
FETAL INCIDENCE	N(%)	0(0.0)	24(15.7)**
HIND LIMB: TIBIA, BENT (M)			
LITTER INCIDENCE	N(%)	0(0.0)	2(10.5)
FETAL INCIDENCE	N(%)	0(0.0)	4(2.6)**
HIND LIMB: FIBULA, BENT (M)			
LITTER INCIDENCE	N(%)	0(0.0)	4(21.0)**
FETAL INCIDENCE	N(%)	0(0.0)	5(3.3)**
HIND LIMB: FIBULA, NOT OSSIFIED (M)			
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)
FETAL INCIDENCE	N(%)	0(0.0)	3(2.0)**
HIND LIMB: PHALANX, SMALL (V)			
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)
HIND LIMB: SHORT (M)			
LITTER INCIDENCE	N(%)	0(0.0)	4(21.0)**
FETAL INCIDENCE	N(%)	0(0.0)	13(8.5)**
HIND LIMB: PHALANGES, FUSED (M)			
LITTER INCIDENCE	N(%)	0(0.0)	4(21.0)**
FETAL INCIDENCE	N(%)	0(0.0)	5(3.3)**
HIND LIMB: NOT OSSIFIED (M)			
LITTER INCIDENCE	N(%)	0(0.0)	2(10.5)
FETAL INCIDENCE	N(%)	0(0.0)	4(2.6)**

(M) = MALFORMATION (V) = VARIATION
** Significantly different from the control group value ($p \leq 0.01$).

TABLE 11 (PAGE 16): FETAL SKELETAL ALTERATIONS - CAESAREAN-DELIVERED LIVE FETUSES (DAY 29 OF GESTATION) - SUMMARY
(See footnotes on the last page of this table.)

GROUP		2	5
TEST MATERIALS		rAvPAL-PEG/ CONTROL ARTICLE	rAvPAL-PEG/ CONTROL ARTICLE
DOSE LEVELS (MG/KG)		2/0	5/0
DOSING (DAYS OF PRESUMED GESTATION)		7-12/13-20	7-12/13-20
LITTERS EVALUATED	N	19	19
LITTERS WITH LIVE FETUS(ES)	N	19	19
FETUSES EVALUATED a	N	173	154
LIVE	N	173	153
DEAD b	N	0	1
HIND LIMB: TIBIA AND FIBULA, MALPOSITIONED (M)			
LITTER INCIDENCE	N(%)	0(0.0)	2(10.5)
FETAL INCIDENCE	N(%)	0(0.0)	4(2.6)**
HIND LIMB: DIGIT, SHORT (M)			
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)
HIND LIMB: PHALANX, ABSENT (M)			
LITTER INCIDENCE	N(%)	0(0.0)	3(15.8)**
FETAL INCIDENCE	N(%)	0(0.0)	3(2.0)**
CAUDAL VERTEBRAE: 12 PRESENT (M)			
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)
FETAL INCIDENCE	N(%)	0(0.0)	3(2.0)**

(M) = MALFORMATION (V) = VARIATION
** Significantly different from the control group value ($p \leq 0.01$).

TABLE 11 (PAGE 17): FETAL SKELETAL ALTERATIONS - CAESAREAN-DELIVERED LIVE FETUSES (DAY 29 OF GESTATION) - SUMMARY
(See footnotes on the last page of this table.)

GROUP		3	6
TEST MATERIALS		CONTROL ARTICLE/ rAvPAL-PEG	CONTROL ARTICLE/ rAvPAL-PEG
DOSE LEVELS (MG/KG)		0/2	0/5
DOSING (DAYS OF PRESUMED GESTATION)		7-10,17-20/11-16	7-10/17-20/11-16
LITTERS EVALUATED	N	19	19
LITTERS WITH LIVE FETUS(ES)	N	19	19
FETUSES EVALUATED a	N	162	160
LIVE	N	162	159
DEAD b	N	0	1
SKULL: ANTERIOR FONTANELLE, IRREGULARLY SHAPED (M)			
LITTER INCIDENCE	N(%)	6(31.6)	11(57.9)**
FETAL INCIDENCE	N(%)	33(20.4)	78(49.0)**
SKULL: NASALS, MIDLINE SUTURE DISPLACED (V)			
LITTER INCIDENCE	N(%)	1(5.3)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)	0(0.0)
SKULL: INTERPARIETALS, INCOMPLETELY OSSIFIED (V)			
LITTER INCIDENCE	N(%)	2(10.5)	14(73.7)**
FETAL INCIDENCE	N(%)	3(1.8)	32(20.1)**
SKULL: PALATE, INCOMPLETELY OSSIFIED (M)			
LITTER INCIDENCE	N(%)	2(10.5)	16(84.2)**
FETAL INCIDENCE	N(%)	6(3.7)	93(58.5)**
SKULL: INTERPARIETALS, NOT OSSIFIED (M)			
LITTER INCIDENCE	N(%)	0(0.0)	18(94.7)**
FETAL INCIDENCE	N(%)	0(0.0)	77(48.4)**
SKULL: FRONTALS, SUTURE IRREGULAR (V)			
LITTER INCIDENCE	N(%)	0(0.0)	8(42.1)**
FETAL INCIDENCE	N(%)	0(0.0)	25(15.7)**
SKULL: FRONTAL, INCOMPLETELY OSSIFIED (V)			
LITTER INCIDENCE	N(%)	0(0.0)	13(68.4)**
FETAL INCIDENCE	N(%)	0(0.0)	70(44.0)**
SKULL: PREMAXILLA, INCOMPLETELY OSSIFIED (M)			
LITTER INCIDENCE	N(%)	0(0.0)	3(15.8)**
FETAL INCIDENCE	N(%)	0(0.0)	14(8.8)**

(M) = MALFORMATION (V) = VARIATION
** Significantly different from the control group value ($p \leq 0.01$).

TABLE 11 (PAGE 18): FETAL SKELETAL ALTERATIONS - CAESAREAN-DELIVERED LIVE FETUSES (DAY 29 OF GESTATION) - SUMMARY
(See footnotes on the last page of this table.)

GROUP		3	6
TEST MATERIALS		CONTROL ARTICLE/ rAvPAL-PEG	CONTROL ARTICLE/ rAvPAL-PEG
DOSE LEVELS (MG/KG)		0/2	0/5
DOSING (DAYS OF PRESUMED GESTATION)		7-10,17-20/11-16	7-10/17-20/11-16
LITTERS EVALUATED	N	19	19
LITTERS WITH LIVE FETUS(ES)	N	19	19
FETUSES EVALUATED a	N	162	160
LIVE	N	162	159
DEAD b	N	0	1
SKULL: FRONTALS, CONTAIN AN INTERFRONTAL (V)			
LITTER INCIDENCE	N(%)	0(0.0)	2(10.5)
FETAL INCIDENCE	N(%)	0(0.0)	2(1.2)
SKULL: NASAL, INCOMPLETELY OSSIFIED (V)			
LITTER INCIDENCE	N(%)	0(0.0)	12(63.2)**
FETAL INCIDENCE	N(%)	0(0.0)	56(35.2)**
SKULL: FRONTALS, SUTURE LARGE (V)			
LITTER INCIDENCE	N(%)	0(0.0)	8(42.1)**
FETAL INCIDENCE	N(%)	0(0.0)	14(8.8)**
SKULL: SUPRAOCCIPITALS, IRREGULARLY SHAPED (V)			
LITTER INCIDENCE	N(%)	0(0.0)	14(73.7)**
FETAL INCIDENCE	N(%)	0(0.0)	43(27.0)**
SKULL: FRONTAL, CONTAINS A INTERFRONTAL (V)			
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)
SKULL: SUPRAOCCIPITALS, INCOMPLETELY OSSIFIED (V)			
LITTER INCIDENCE	N(%)	0(0.0)	7(36.8)**
FETAL INCIDENCE	N(%)	0(0.0)	15(9.4)**
SKULL: SUPRAOCCIPITALS, NOT OSSIFIED (M)			
LITTER INCIDENCE	N(%)	0(0.0)	3(15.8)**
FETAL INCIDENCE	N(%)	0(0.0)	3(1.9)**
SKULL: MANDIBLES, SHORT (M)			
LITTER INCIDENCE	N(%)	0(0.0)	8(42.1)**
FETAL INCIDENCE	N(%)	0(0.0)	44(27.7)**

(M) = MALFORMATION (V) = VARIATION
** Significantly different from the control group value ($p \leq 0.01$).

TABLE 11 (PAGE 19): FETAL SKELETAL ALTERATIONS - CAESAREAN-DELIVERED LIVE FETUSES (DAY 29 OF GESTATION) - SUMMARY
(See footnotes on the last page of this table.)

GROUP		3	6
TEST MATERIALS		CONTROL ARTICLE/ rAvPAL-PEG	CONTROL ARTICLE/ rAvPAL-PEG
DOSE LEVELS (MG/KG)		0/2	0/5
DOSING (DAYS OF PRESUMED GESTATION)		7-10,17-20/11-16	7-10/17-20/11-16
LITTERS EVALUATED	N	19	19
LITTERS WITH LIVE FETUS(ES)	N	19	19
FETUSES EVALUATED a	N	162	160
LIVE	N	162	159
DEAD b	N	0	1
SKULL: ANTERIOR FONTANELLE, LARGE (M)			
LITTER INCIDENCE	N(%)	0(0.0)	10(52.6)**
FETAL INCIDENCE	N(%)	0(0.0)	66(41.5)**
SKULL: FRONTAL, CONTAINS AN INTRAFRONTAL (V)			
LITTER INCIDENCE	N(%)	1(5.3)	6(31.6)**
FETAL INCIDENCE	N(%)	1(0.6)	10(6.3)**
SKULL: NASAL, SMALL (V)			
LITTER INCIDENCE	N(%)	0(0.0)	2(10.5)
FETAL INCIDENCE	N(%)	0(0.0)	2(1.2)
SKULL: NASAL, IRREGULARLY SHAPED (V)			
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)
FETAL INCIDENCE	N(%)	0(0.0)	2(1.2)
SKULL: EYE SOCKET, SMALL (M)			
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)
SKULL: PARIETAL, INCOMPLETELY OSSIFIED (V)			
LITTER INCIDENCE	N(%)	0(0.0)	5(26.3)**
FETAL INCIDENCE	N(%)	0(0.0)	15(9.4)**
SKULL: MAXILLAE, SHORT (M)			
LITTER INCIDENCE	N(%)	0(0.0)	6(31.6)**
FETAL INCIDENCE	N(%)	0(0.0)	41(25.8)**
SKULL: PARIETAL, CONTAINS A HOLE (V)			
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)

(M) = MALFORMATION (V) = VARIATION
** Significantly different from the control group value ($p \leq 0.01$).

TABLE 11 (PAGE 20): FETAL SKELETAL ALTERATIONS - CAESAREAN-DELIVERED LIVE FETUSES (DAY 29 OF GESTATION) - SUMMARY
(See footnotes on the last page of this table.)

GROUP			3	6
TEST MATERIALS			CONTROL ARTICLE/ rAvPAL-PEG	CONTROL ARTICLE/ rAvPAL-PEG
DOSE LEVELS (MG/KG)			0/2	0/5
DOSING (DAYS OF PRESUMED GESTATION)			7-10,17-20/11-16	7-10/17-20/11-16
LITTERS EVALUATED		N	19	19
LITTERS WITH LIVE FETUS(ES)		N	19	19
FETUSES EVALUATED a		N	162	160
LIVE		N	162	159
DEAD b		N	0	1
SKULL: NOT OSSIFIED (M)				
LITTER INCIDENCE		N(%)	0(0.0)	3(15.8)**
FETAL INCIDENCE		N(%)	0(0.0)	5(3.1)**
SKULL: INCOMPLETELY OSSIFIED (M)				
LITTER INCIDENCE		N(%)	0(0.0)	3(15.8)**
FETAL INCIDENCE		N(%)	0(0.0)	24(15.1)**
HYOID: ALA, ANGULATED (V)				
LITTER INCIDENCE		N(%)	4(21.0)	6(31.6)**
FETAL INCIDENCE		N(%)	8(4.9)	20(12.6)**
HYOID: ALA, SHORT (V)				
LITTER INCIDENCE		N(%)	0(0.0)	13(68.4)**
FETAL INCIDENCE		N(%)	0(0.0)	65(40.9)**
HYOID: ALA, NOT OSSIFIED (V)				
LITTER INCIDENCE		N(%)	0(0.0)	5(26.3)**
FETAL INCIDENCE		N(%)	0(0.0)	16(10.1)**
HYOID: ALA, INCOMPLETELY OSSIFIED (V)				
LITTER INCIDENCE		N(%)	0(0.0)	1(5.3)
FETAL INCIDENCE		N(%)	0(0.0)	1(0.6)
HYOID: ALA, SMALL (V)				
LITTER INCIDENCE		N(%)	0(0.0)	1(5.3)
FETAL INCIDENCE		N(%)	0(0.0)	2(1.2)
CERVICAL VERTEBRAE: CENTRUM, BIFID (V)				
LITTER INCIDENCE		N(%)	0(0.0)	1(5.3)
FETAL INCIDENCE		N(%)	0(0.0)	1(0.6)

(M) = MALFORMATION (V) = VARIATION
** Significantly different from the control group value ($p \leq 0.01$).

TABLE 11 (PAGE 21): FETAL SKELETAL ALTERATIONS - CAESAREAN-DELIVERED LIVE FETUSES (DAY 29 OF GESTATION) - SUMMARY
(See footnotes on the last page of this table.)

GROUP		3	6
TEST MATERIALS		CONTROL ARTICLE/ rAvPAL-PEG	CONTROL ARTICLE/ rAvPAL-PEG
DOSE LEVELS (MG/KG)		0/2	0/5
DOSING (DAYS OF PRESUMED GESTATION)		7-10,17-20/11-16	7-10/17-20/11-16
LITTERS EVALUATED	N	19	19
LITTERS WITH LIVE FETUS(ES)	N	19	19
FETUSES EVALUATED a	N	162	160
LIVE	N	162	159
DEAD b	N	0	1
CERVICAL VERTEBRAE: CENTRA, FUSED (M)			
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)
THORACIC VERTEBRAE: CENTRUM, UNILATERAL OSSIFICATION (V)			
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)
CAUDAL VERTEBRAE: SPACE (M)			
LITTER INCIDENCE	N(%)	1(5.3)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)	0(0.0)
CAUDAL VERTEBRAE: MISALIGNED (V)			
LITTER INCIDENCE	N(%)	1(5.3)	2(10.5)
FETAL INCIDENCE	N(%)	1(0.6)	2(1.2)
CAUDAL VERTEBRAE: FUSED (M)			
LITTER INCIDENCE	N(%)	0(0.0)	2(10.5)
FETAL INCIDENCE	N(%)	0(0.0)	2(1.2)
CAUDAL VERTEBRAE: 15 PRESENT (V)			
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)
RIBS: SHORT (V)			
LITTER INCIDENCE	N(%)	1(5.3)	19(100.0)**
FETAL INCIDENCE	N(%)	9(5.6)	124(78.0)**
RIBS: THICKENED (V)			
LITTER INCIDENCE	N(%)	1(5.3)	15(78.9)**
FETAL INCIDENCE	N(%)	1(0.6)	31(19.5)**

(M) = MALFORMATION (V) = VARIATION
** Significantly different from the control group value ($p \leq 0.01$).

TABLE 11 (PAGE 22): FETAL SKELETAL ALTERATIONS - CAESAREAN-DELIVERED LIVE FETUSES (DAY 29 OF GESTATION) - SUMMARY
(See footnotes on the last page of this table.)

GROUP			3	6
TEST MATERIALS			CONTROL ARTICLE/ rAvPAL-PEG	CONTROL ARTICLE/ rAvPAL-PEG
DOSE LEVELS (MG/KG)			0/2	0/5
DOSING (DAYS OF PRESUMED GESTATION)			7-10,17-20/11-16	7-10/17-20/11-16
LITTERS EVALUATED		N	19	19
LITTERS WITH LIVE FETUS(ES)		N	19	19
FETUSES EVALUATED a		N	162	160
LIVE		N	162	159
DEAD b		N	0	1
RIBS: BOWED (V)				
LITTER INCIDENCE		N(%)	0(0.0)	5(26.3)**
FETAL INCIDENCE		N(%)	0(0.0)	6(3.8)**
RIBS: INCOMPLETELY OSSIFIED (V)				
LITTER INCIDENCE		N(%)	0(0.0)	2(10.5)
FETAL INCIDENCE		N(%)	0(0.0)	2(1.2)
RIBS: IRREGULARLY SHAPED (V)				
LITTER INCIDENCE		N(%)	0(0.0)	2(10.5)
FETAL INCIDENCE		N(%)	0(0.0)	3(1.9)**
RIBS: FUSED (V)				
LITTER INCIDENCE		N(%)	0(0.0)	1(5.3)
FETAL INCIDENCE		N(%)	0(0.0)	1(0.6)
MANUBRIUM: IRREGULARLY SHAPED (V)				
LITTER INCIDENCE		N(%)	0(0.0)	1(5.3)
FETAL INCIDENCE		N(%)	0(0.0)	1(0.6)
MANUBRIUM: FUSED (V)				
LITTER INCIDENCE		N(%)	0(0.0)	1(5.3)
FETAL INCIDENCE		N(%)	0(0.0)	2(1.2)
STERNAL CENTRA: FUSED (V)				
LITTER INCIDENCE		N(%)	3(15.8)	17(89.5)**
FETAL INCIDENCE		N(%)	13(8.0)	66(41.5)**
STERNAL CENTRA: INCOMPLETELY OSSIFIED (V)				
LITTER INCIDENCE		N(%)	1(5.3)	8(42.1)**
FETAL INCIDENCE		N(%)	3(1.8)	17(10.7)**

(M) = MALFORMATION (V) = VARIATION
** Significantly different from the control group value ($p \leq 0.01$).

TABLE 11 (PAGE 23): FETAL SKELETAL ALTERATIONS - CAESAREAN-DELIVERED LIVE FETUSES (DAY 29 OF GESTATION) - SUMMARY
(See footnotes on the last page of this table.)

GROUP		3	6
TEST MATERIALS		CONTROL ARTICLE/ rAvPAL-PEG	CONTROL ARTICLE/ rAvPAL-PEG
DOSE LEVELS (MG/KG)		0/2	0/5
DOSING (DAYS OF PRESUMED GESTATION)		7-10,17-20/11-16	7-10/17-20/11-16
LITTERS EVALUATED	N	19	19
LITTERS WITH LIVE FETUS(ES)	N	19	19
FETUSES EVALUATED a	N	162	160
LIVE	N	162	159
DEAD b	N	0	1
STERNAL CENTRA: ASYMMETRIC (V)			
LITTER INCIDENCE	N(%)	0(0.0)	2(10.5)
FETAL INCIDENCE	N(%)	0(0.0)	2(1.2)
STERNAL CENTRA: IRREGULARLY SHAPED (V)			
LITTER INCIDENCE	N(%)	0(0.0)	2(10.5)
FETAL INCIDENCE	N(%)	0(0.0)	4(2.5)**
STERNAL CENTRA: LARGE (V)			
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)
FETAL INCIDENCE	N(%)	0(0.0)	2(1.2)
STERNAL CENTRA: NOT OSSIFIED (V)			
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)
XIPHOID: IRREGULARLY SHAPED (V)			
LITTER INCIDENCE	N(%)	0(0.0)	11(57.9)**
FETAL INCIDENCE	N(%)	0(0.0)	21(13.2)**
XIPHOID: FUSED (V)			
LITTER INCIDENCE	N(%)	0(0.0)	6(31.6)**
FETAL INCIDENCE	N(%)	0(0.0)	7(4.4)**
XIPHOID: LARGE (V)			
LITTER INCIDENCE	N(%)	0(0.0)	2(10.5)
FETAL INCIDENCE	N(%)	0(0.0)	4(2.5)**
CLAVICULAE: BENT (M)			
LITTER INCIDENCE	N(%)	0(0.0)	2(10.5)
FETAL INCIDENCE	N(%)	0(0.0)	2(1.2)

(M) = MALFORMATION (V) = VARIATION
** Significantly different from the control group value ($p \leq 0.01$).

TABLE 11 (PAGE 24): FETAL SKELETAL ALTERATIONS - CAESAREAN-DELIVERED LIVE FETUSES (DAY 29 OF GESTATION) - SUMMARY
(See footnotes on the last page of this table.)

GROUP		3	6
TEST MATERIALS		CONTROL ARTICLE/ rAvPAL-PEG	CONTROL ARTICLE/ rAvPAL-PEG
DOSE LEVELS (MG/KG)		0/2	0/5
DOSING (DAYS OF PRESUMED GESTATION)		7-10,17-20/11-16	7-10/17-20/11-16
LITTERS EVALUATED	N	19	19
LITTERS WITH LIVE FETUS(ES)	N	19	19
FETUSES EVALUATED a	N	162	160
LIVE	N	162	159
DEAD b	N	0	1
SCAPULAE: ALA AND BODY, SMALL (V)			
LITTER INCIDENCE	N(%)	3(15.8)	12(63.2)**
FETAL INCIDENCE	N(%)	20(12.3)	88(55.3)**
SCAPULAE: ALA AND BODY, IRREGULARLY SHAPED (V)			
LITTER INCIDENCE	N(%)	0(0.0)	3(15.8)**
FETAL INCIDENCE	N(%)	0(0.0)	6(3.8)**
SCAPULAE: ALA, IRREGULARLY SHAPED (V)			
LITTER INCIDENCE	N(%)	0(0.0)	2(10.5)
FETAL INCIDENCE	N(%)	0(0.0)	2(1.2)
FORE LIMB: RADIUS, SHORT (M)			
LITTER INCIDENCE	N(%)	0(0.0)	8(42.1)**
FETAL INCIDENCE	N(%)	0(0.0)	50(31.4)**
FORE LIMB: HUMERUS, SHORT (M)			
LITTER INCIDENCE	N(%)	0(0.0)	9(47.4)**
FETAL INCIDENCE	N(%)	0(0.0)	58(36.5)**
FORE LIMB: ULNA, SHORT (M)			
LITTER INCIDENCE	N(%)	0(0.0)	7(36.8)**
FETAL INCIDENCE	N(%)	0(0.0)	49(30.8)**
FORE LIMB: HUMERUS, BENT (M)			
LITTER INCIDENCE	N(%)	0(0.0)	2(10.5)
FETAL INCIDENCE	N(%)	0(0.0)	11(6.9)**
FORE LIMB: RADIUS, BENT (M)			
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)

(M) = MALFORMATION (V) = VARIATION
** Significantly different from the control group value ($p \leq 0.01$).

TABLE 11 (PAGE 25): FETAL SKELETAL ALTERATIONS - CAESAREAN-DELIVERED LIVE FETUSES (DAY 29 OF GESTATION) - SUMMARY
(See footnotes on the last page of this table.)

GROUP		3	6
TEST MATERIALS		CONTROL ARTICLE/ rAvPAL-PEG	CONTROL ARTICLE/ rAvPAL-PEG
DOSE LEVELS (MG/KG)		0/2	0/5
DOSING (DAYS OF PRESUMED GESTATION)		7-10,17-20/11-16	7-10/17-20/11-16
LITTERS EVALUATED	N	19	19
LITTERS WITH LIVE FETUS(ES)	N	19	19
FETUSES EVALUATED a	N	162	160
LIVE	N	162	159
DEAD b	N	0	1
FORE LIMB: SHORT (M)			
LITTER INCIDENCE	N(%)	0(0.0)	5(26.3)**
FETAL INCIDENCE	N(%)	0(0.0)	30(18.9)**
PELVIS: PUBIS, NOT OSSIFIED (V)			
LITTER INCIDENCE	N(%)	0(0.0)	7(36.8)**
FETAL INCIDENCE	N(%)	0(0.0)	15(9.4)**
PELVIS: ILIUM, SHORT (M)			
LITTER INCIDENCE	N(%)	0(0.0)	10(52.6)**
FETAL INCIDENCE	N(%)	0(0.0)	34(21.4)**
PELVIS: PUBIS, CLOSE SET (V)			
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)
HIND LIMB: TIBIA, NOT OSSIFIED (M)			
LITTER INCIDENCE	N(%)	0(0.0)	7(36.8)**
FETAL INCIDENCE	N(%)	0(0.0)	14(8.8)**
HIND LIMB: TIBIA, SHORT (M)			
LITTER INCIDENCE	N(%)	0(0.0)	8(42.1)**
FETAL INCIDENCE	N(%)	0(0.0)	29(18.2)**
HIND LIMB: FIBULA, BENT (M)			
LITTER INCIDENCE	N(%)	0(0.0)	3(15.8)**
FETAL INCIDENCE	N(%)	0(0.0)	6(3.8)**
HIND LIMB: FIBULA, SHORT (M)			
LITTER INCIDENCE	N(%)	0(0.0)	8(42.1)**
FETAL INCIDENCE	N(%)	0(0.0)	29(18.2)**

(M) = MALFORMATION (V) = VARIATION
** Significantly different from the control group value ($p \leq 0.01$).

TABLE 11 (PAGE 26): FETAL SKELETAL ALTERATIONS - CAESAREAN-DELIVERED LIVE FETUSES (DAY 29 OF GESTATION) - SUMMARY
(See footnotes on the last page of this table.)

GROUP		3	6
TEST MATERIALS		CONTROL ARTICLE/ rAvPAL-PEG	CONTROL ARTICLE/ rAvPAL-PEG
DOSE LEVELS (MG/KG)		0/2	0/5
DOSING (DAYS OF PRESUMED GESTATION)		7-10,17-20/11-16	7-10/17-20/11-16
LITTERS EVALUATED	N	19	19
LITTERS WITH LIVE FETUS(ES)	N	19	19
FETUSES EVALUATED a	N	162	160
LIVE	N	162	159
DEAD b	N	0	1
HIND LIMB: FEMUR, SHORT (M)			
LITTER INCIDENCE	N(%)	0(0.0)	7(36.8)**
FETAL INCIDENCE	N(%)	0(0.0)	30(18.9)**
HIND LIMB: TIBIA AND FIBULA, FUSED (M)			
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)
FETAL INCIDENCE	N(%)	0(0.0)	4(2.5)**
HIND LIMB: TIBIA, BENT (M)			
LITTER INCIDENCE	N(%)	0(0.0)	6(31.6)**
FETAL INCIDENCE	N(%)	0(0.0)	10(6.3)**
HIND LIMB: FEMUR, BENT (M)			
LITTER INCIDENCE	N(%)	0(0.0)	2(10.5)
FETAL INCIDENCE	N(%)	0(0.0)	3(1.9)**
HIND LIMB: TIBIA AND FIBULA, MALPOSTIONED (M)			
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)
HIND LIMB: SHORT (M)			
LITTER INCIDENCE	N(%)	0(0.0)	4(21.0)**
FETAL INCIDENCE	N(%)	0(0.0)	29(18.2)**
HIND LIMB: FIBULA, NOT OSSIFIED (M)			
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)

(M) = MALFORMATION (V) = VARIATION

TABLE 11 (PAGE 27): FETAL SKELETAL ALTERATIONS - CAESAREAN-DELIVERED LIVE FETUSES (DAY 29 OF GESTATION) - SUMMARY
(See footnotes on the last page of this table.)

GROUP		3	6
TEST MATERIALS		CONTROL ARTICLE/ rAvPAL-PEG	CONTROL ARTICLE/ rAvPAL-PEG
DOSE LEVELS (MG/KG)		0/2	0/5
DOSING (DAYS OF PRESUMED GESTATION)		7-10,17-20/11-16	7-10/17-20/11-16
LITTERS EVALUATED	N	19	19
LITTERS WITH LIVE FETUS(ES)	N	19	19
FETUSES EVALUATED a	N	162	160
LIVE	N	162	159
DEAD b	N	0	1
HIND LIMB: NOT OSSIFIED (M)			
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.6)
HIND LIMB: BENT (M)			
LITTER INCIDENCE	N(%)	0(0.0)	1(5.3)
FETAL INCIDENCE	N(%)	0(0.0)	2(1.2)

(M) = MALFORMATION (V) = VARIATION
** Significantly different from the control group value ($p \leq 0.01$).

TABLE 11 (PAGE 28): FETAL SKELETAL ALTERATIONS - CAESAREAN-DELIVERED LIVE FETUSES (DAY 29 OF GESTATION) - SUMMARY
(See footnotes on the last page of this table.)

GROUP		4	7
TEST MATERIALS		CONTROL ARTICLE/ rAvPAL-PEG	CONTROL ARTICLE/ rAvPAL-PEG
DOSE LEVELS (MG/KG)		0/2	0/5
DOSING (DAYS OF PRESUMED GESTATION)		7-14/15-20	7-14/15-20
LITTERS EVALUATED	N	20	15
LITTERS WITH LIVE FETUS(ES)	N	20	15
FETUSES EVALUATED a	N	163	139
LIVE	N	163	139
SKULL: INTERPARIETALS, INCOMPLETELY OSSIFIED (V)			
LITTER INCIDENCE	N(%)	3(15.0)	12(80.0)**
FETAL INCIDENCE	N(%)	5(3.1)	53(38.1)**
SKULL: NASALS, MIDLINE SUTURE DISPLACED (V)			
LITTER INCIDENCE	N(%)	1(5.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)	0(0.0)
SKULL: ANTERIOR FONTANELLE, IRREGULARLY SHAPED (V)			
LITTER INCIDENCE	N(%)	1(5.0)	13(86.7)**
FETAL INCIDENCE	N(%)	1(0.6)	80(57.6)**
SKULL: EYE SOCKET, SMALL (M)			
LITTER INCIDENCE	N(%)	2(10.0)	1(6.7)
FETAL INCIDENCE	N(%)	3(1.8)	1(0.7)
SKULL: INCOMPLETELY OSSIFIED (M)			
LITTER INCIDENCE	N(%)	1(5.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)	0(0.0)
SKULL: MANDIBLE, SHORT (M)			
LITTER INCIDENCE	N(%)	1(5.0)	9(60.0)**
FETAL INCIDENCE	N(%)	1(0.6)	59(42.4)**
SKULL: ANTERIOR FONTANELLE, LARGE (M)			
LITTER INCIDENCE	N(%)	0(0.0)	13(86.7)**
FETAL INCIDENCE	N(%)	0(0.0)	120(86.3)**
SKULL: PARIETAL, CONTAINS A HOLE (V)			
LITTER INCIDENCE	N(%)	0(0.0)	2(13.3)
FETAL INCIDENCE	N(%)	0(0.0)	2(1.4)

(M) = MALFORMATION (V) = VARIATION
** Significantly different from the control group value ($p \leq 0.01$).

TABLE 11 (PAGE 29): FETAL SKELETAL ALTERATIONS - CAESAREAN-DELIVERED LIVE FETUSES (DAY 29 OF GESTATION) - SUMMARY
(See footnotes on the last page of this table.)

GROUP		4	7
TEST MATERIALS		CONTROL ARTICLE/ rAvPAL-PEG	CONTROL ARTICLE/ rAvPAL-PEG
DOSE LEVELS (MG/KG)		0/2	0/5
DOSING (DAYS OF PRESUMED GESTATION)		7-14/15-20	7-14/15-20
LITTERS EVALUATED	N	20	15
LITTERS WITH LIVE FETUS(ES)	N	20	15
FETUSES EVALUATED a	N	163	139
LIVE	N	163	139
SKULL: FRONTAL, INCOMPLETELY OSSIFIED (V)			
LITTER INCIDENCE	N(%)	0(0.0)	10(66.7)**
FETAL INCIDENCE	N(%)	0(0.0)	52(37.4)**
SKULL: NASAL, SHORT (M)			
LITTER INCIDENCE	N(%)	0(0.0)	9(60.0)**
FETAL INCIDENCE	N(%)	0(0.0)	59(42.4)**
SKULL: MAXILLA, SHORT (M)			
LITTER INCIDENCE	N(%)	0(0.0)	9(60.0)**
FETAL INCIDENCE	N(%)	0(0.0)	59(42.4)**
SKULL: EXOCCIPITAL, SMALL (V)			
LITTER INCIDENCE	N(%)	0(0.0)	3(20.0)**
FETAL INCIDENCE	N(%)	0(0.0)	14(10.1)**
SKULL: FRONTALS, CONTAIN AN INTERFRONTAL (V)			
LITTER INCIDENCE	N(%)	0(0.0)	1(6.7)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.7)
SKULL: NASAL, INCOMPLETELY OSSIFIED (V)			
LITTER INCIDENCE	N(%)	0(0.0)	5(33.3)**
FETAL INCIDENCE	N(%)	0(0.0)	23(16.5)**
SKULL: INTERPARIETALS, NOT OSSIFIED (V)			
LITTER INCIDENCE	N(%)	0(0.0)	3(20.0)**
FETAL INCIDENCE	N(%)	0(0.0)	10(7.2)**
SKULL: FRONTALS, FUSED (V)			
LITTER INCIDENCE	N(%)	0(0.0)	1(6.7)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.7)

(M) = MALFORMATION (V) = VARIATION
** Significantly different from the control group value ($p \leq 0.01$).

TABLE 11 (PAGE 30): FETAL SKELETAL ALTERATIONS - CAESAREAN-DELIVERED LIVE FETUSES (DAY 29 OF GESTATION) - SUMMARY
(See footnotes on the last page of this table.)

GROUP		4	7
TEST MATERIALS		CONTROL ARTICLE/ rAvPAL-PEG	CONTROL ARTICLE/ rAvPAL-PEG
DOSE LEVELS (MG/KG)		0/2	0/5
DOSING (DAYS OF PRESUMED GESTATION)		7-14/15-20	7-14/15-20
LITTERS EVALUATED	N	20	15
LITTERS WITH LIVE FETUS(ES)	N	20	15
FETUSES EVALUATED a	N	163	139
LIVE	N	163	139
SKULL: PALATE, INCOMPLETELY OSSIFIED (M)			
LITTER INCIDENCE	N(%)	0(0.0)	1(6.7)
FETAL INCIDENCE	N(%)	0(0.0)	3(2.2)**
SKULL: NASAL, CONTAINS AN INTRANASAL (V)			
LITTER INCIDENCE	N(%)	0(0.0)	1(6.7)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.7)
SKULL: PARIETAL, CONTAINS AN INTRAPARIETAL (V)			
LITTER INCIDENCE	N(%)	0(0.0)	1(6.7)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.7)
HYOID: ALA, ANGULATED (V)			
LITTER INCIDENCE	N(%)	4(20.0)	0(0.0)
FETAL INCIDENCE	N(%)	7(4.3)	0(0.0)
HYOID: ALA, SHORT (V)			
LITTER INCIDENCE	N(%)	0(0.0)	8(53.3)**
FETAL INCIDENCE	N(%)	0(0.0)	38(27.3)**
HYOID: NOT OSSIFIED (V)			
LITTER INCIDENCE	N(%)	0(0.0)	4(26.7)**
FETAL INCIDENCE	N(%)	0(0.0)	5(3.6)**
HYOID: ALA, NOT OSSIFIED (V)			
LITTER INCIDENCE	N(%)	0(0.0)	9(60.0)**
FETAL INCIDENCE	N(%)	0(0.0)	32(23.0)**
HYOID: ALA, SMALL (V)			
LITTER INCIDENCE	N(%)	0(0.0)	9(60.0)**
FETAL INCIDENCE	N(%)	0(0.0)	21(15.1)**

(M) = MALFORMATION (V) = VARIATION
** Significantly different from the control group value ($p \leq 0.01$).

TABLE 11 (PAGE 31): FETAL SKELETAL ALTERATIONS - CAESAREAN-DELIVERED LIVE FETUSES (DAY 29 OF GESTATION) - SUMMARY
(See footnotes on the last page of this table.)

GROUP		4	7
TEST MATERIALS		CONTROL ARTICLE/ rAvPAL-PEG	CONTROL ARTICLE/ rAvPAL-PEG
DOSE LEVELS (MG/KG)		0/2	0/5
DOSING (DAYS OF PRESUMED GESTATION)		7-14/15-20	7-14/15-20
LITTERS EVALUATED	N	20	15
LITTERS WITH LIVE FETUS(ES)	N	20	15
FETUSES EVALUATED a	N	163	139
LIVE	N	163	139
CAUDAL VERTEBRAE: MISALIGNED (V)			
LITTER INCIDENCE	N(%)	2(10.0)	0(0.0)
FETAL INCIDENCE	N(%)	2(1.2)	0(0.0)
RIBS: SHORT (V)			
LITTER INCIDENCE	N(%)	5(25.0)	15(100.0)**
FETAL INCIDENCE	N(%)	14(8.6)	127(91.4)**
RIBS: BOWED (V)			
LITTER INCIDENCE	N(%)	3(15.0)	1(6.7)
FETAL INCIDENCE	N(%)	3(1.8)	1(0.7)
RIBS: WAVY (V)			
LITTER INCIDENCE	N(%)	1(5.0)	1(6.7)
FETAL INCIDENCE	N(%)	1(0.6)	1(0.7)
RIBS: IRREGULARLY SHAPED (V)			
LITTER INCIDENCE	N(%)	0(0.0)	9(60.0)**
FETAL INCIDENCE	N(%)	0(0.0)	27(19.4)**
RIBS: THICKENED (V)			
LITTER INCIDENCE	N(%)	0(0.0)	1(6.7)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.7)
MANUBRIUM: IRREGULARLY SHAPED (V)			
LITTER INCIDENCE	N(%)	1(5.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)	0(0.0)
STERNAL CENTRA: FUSED (V)			
LITTER INCIDENCE	N(%)	3(15.0)	0(0.0)
FETAL INCIDENCE	N(%)	6(3.7)	0(0.0)

(M) = MALFORMATION (V) = VARIATION
** Significantly different from the control group value ($p \leq 0.01$).

TABLE 11 (PAGE 32): FETAL SKELETAL ALTERATIONS - CAESAREAN-DELIVERED LIVE FETUSES (DAY 29 OF GESTATION) - SUMMARY
(See footnotes on the last page of this table.)

GROUP		4	7
TEST MATERIALS		CONTROL ARTICLE/ rAvPAL-PEG	CONTROL ARTICLE/ rAvPAL-PEG
DOSE LEVELS (MG/KG)		0/2	0/5
DOSING (DAYS OF PRESUMED GESTATION)		7-14/15-20	7-14/15-20
LITTERS EVALUATED	N	20	15
LITTERS WITH LIVE FETUS(ES)	N	20	15
FETUSES EVALUATED a	N	163	139
LIVE	N	163	139
STERNAL CENTRA: ASYMMETRIC (V)			
LITTER INCIDENCE	N(%)	1(5.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)	0(0.0)
STERNAL CENTRA: INCOMPLETELY OSSIFIED (V)			
LITTER INCIDENCE	N(%)	0(0.0)	6(40.0)**
FETAL INCIDENCE	N(%)	0(0.0)	12(8.6)**
STERNAL CENTRA: NOT OSSIFIED (V)			
LITTER INCIDENCE	N(%)	0(0.0)	2(13.3)
FETAL INCIDENCE	N(%)	0(0.0)	2(1.4)
CLAVICULAE: INCOMPLETELY OSSIFIED (V)			
LITTER INCIDENCE	N(%)	1(5.0)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)	0(0.0)
SCAPULAE: ALA AND BODY, SMALL (V)			
LITTER INCIDENCE	N(%)	4(20.0)	15(100.0)**
FETAL INCIDENCE	N(%)	11(6.7)	137(98.6)**
SCAPULAE: ALA AND BODY, IRREGULARLY SHAPED (V)			
LITTER INCIDENCE	N(%)	1(5.0)	2(13.3)
FETAL INCIDENCE	N(%)	1(0.6)	4(2.9)**
SCAPULAE: BODY, IRREGULARLY SHAPED (V)			
LITTER INCIDENCE	N(%)	0(0.0)	15(100.0)**
FETAL INCIDENCE	N(%)	0(0.0)	91(65.5)**
FORE LIMB: BENT (M)			
LITTER INCIDENCE	N(%)	1(5.0)	1(6.7)
FETAL INCIDENCE	N(%)	1(0.6)	6(4.3)**

(M) = MALFORMATION (V) = VARIATION
** Significantly different from the control group value ($p \leq 0.01$).

TABLE 11 (PAGE 33): FETAL SKELETAL ALTERATIONS - CAESAREAN-DELIVERED LIVE FETUSES (DAY 29 OF GESTATION) - SUMMARY
(See footnotes on the last page of this table.)

GROUP		4	7
TEST MATERIALS		CONTROL ARTICLE/ rAvPAL-PEG	CONTROL ARTICLE/ rAvPAL-PEG
DOSE LEVELS (MG/KG)		0/2	0/5
DOSING (DAYS OF PRESUMED GESTATION)		7-14/15-20	7-14/15-20
LITTERS EVALUATED	N	20	15
LITTERS WITH LIVE FETUS(ES)	N	20	15
FETUSES EVALUATED a	N	163	139
LIVE	N	163	139
FORE LIMB: SHORT (M)			
LITTER INCIDENCE	N(%)	0(0.0)	2(13.3)
FETAL INCIDENCE	N(%)	0(0.0)	8(5.8)**
FORE LIMB: HUMERUS, SHORT (M)			
LITTER INCIDENCE	N(%)	0(0.0)	10(66.7)**
FETAL INCIDENCE	N(%)	0(0.0)	87(62.6)**
FORE LIMB: RADIUS, SHORT (M)			
LITTER INCIDENCE	N(%)	0(0.0)	10(66.7)**
FETAL INCIDENCE	N(%)	0(0.0)	88(63.3)**
FORE LIMB: ULNA, SHORT (M)			
LITTER INCIDENCE	N(%)	0(0.0)	10(66.7)**
FETAL INCIDENCE	N(%)	0(0.0)	88(63.3)**
FORE LIMB: HUMERUS, BENT (M)			
LITTER INCIDENCE	N(%)	0(0.0)	1(6.7)
FETAL INCIDENCE	N(%)	0(0.0)	1(0.7)
FORE LIMB: RADIUS, BENT (M)			
LITTER INCIDENCE	N(%)	0(0.0)	4(26.7)**
FETAL INCIDENCE	N(%)	0(0.0)	10(7.2)**
FORE LIMB: ULNA, BENT (M)			
LITTER INCIDENCE	N(%)	0(0.0)	5(33.3)**
FETAL INCIDENCE	N(%)	0(0.0)	16(11.5)**
PELVIS: ILIUM, SHORT (M)			
LITTER INCIDENCE	N(%)	0(0.0)	11(73.3)**
FETAL INCIDENCE	N(%)	0(0.0)	64(46.0)**

(M) = MALFORMATION (V) = VARIATION
** Significantly different from the control group value ($p \leq 0.01$).

TABLE 11 (PAGE 34): FETAL SKELETAL ALTERATIONS - CAESAREAN-DELIVERED LIVE FETUSES (DAY 29 OF GESTATION) - SUMMARY
(See footnotes on the last page of this table.)

GROUP		4	7
TEST MATERIALS		CONTROL ARTICLE/ rAvPAL-PEG	CONTROL ARTICLE/ rAvPAL-PEG
DOSE LEVELS (MG/KG)		0/2	0/5
DOSING (DAYS OF PRESUMED GESTATION)		7-14/15-20	7-14/15-20
LITTERS EVALUATED	N	20	15
LITTERS WITH LIVE FETUS(ES)	N	20	15
FETUSES EVALUATED a	N	163	139
LIVE	N	163	139
PELVIS: PUBIS, NOT OSSIFIED (V)			
LITTER INCIDENCE	N(%)	0(0.0)	3(20.0)**
FETAL INCIDENCE	N(%)	0(0.0)	3(2.2)**
HIND LIMB: BENT (M)			
LITTER INCIDENCE	N(%)	1(5.0)	5(33.3)**
FETAL INCIDENCE	N(%)	1(0.6)	22(15.8)**
HIND LIMB: FEMUR, SHORT (M)			
LITTER INCIDENCE	N(%)	0(0.0)	10(66.7)**
FETAL INCIDENCE	N(%)	0(0.0)	91(65.5)**
HIND LIMB: FIBULA, SHORT (M)			
LITTER INCIDENCE	N(%)	0(0.0)	10(66.7)**
FETAL INCIDENCE	N(%)	0(0.0)	94(67.6)**
HIND LIMB: TIBIA, SHORT (M)			
LITTER INCIDENCE	N(%)	0(0.0)	10(66.7)**
FETAL INCIDENCE	N(%)	0(0.0)	92(66.2)**
HIND LIMB: FEMUR, BENT (M)			
LITTER INCIDENCE	N(%)	1(5.0)	10(66.7)**
FETAL INCIDENCE	N(%)	1(0.6)	71(51.1)**
HIND LIMB: FIBULA, BENT (M)			
LITTER INCIDENCE	N(%)	1(5.0)	10(66.7)**
FETAL INCIDENCE	N(%)	1(0.6)	80(57.6)**
HIND LIMB: TIBIA, BENT (M)			
LITTER INCIDENCE	N(%)	1(5.0)	10(66.7)**
FETAL INCIDENCE	N(%)	1(0.6)	80(57.6)**

(M) = MALFORMATION (V) = VARIATION
** Significantly different from the control group value ($p \leq 0.01$).

TABLE 11 (PAGE 35): FETAL SKELETAL ALTERATIONS - CAESAREAN-DELIVERED LIVE FETUSES (DAY 29 OF GESTATION) - SUMMARY
(See footnotes on the last page of this table.)

GROUP		4	7
TEST MATERIALS		CONTROL ARTICLE/ rAvPAL-PEG	CONTROL ARTICLE/ rAvPAL-PEG
DOSE LEVELS (MG/KG)		0/2	0/5
DOSING (DAYS OF PRESUMED GESTATION)		7-14/15-20	7-14/15-20
LITTERS EVALUATED	N	20	15
LITTERS WITH LIVE FETUS(ES)	N	20	15
FETUSES EVALUATED a	N	163	139
LIVE	N	163	139
HIND LIMB: SHORT (M)			
LITTER INCIDENCE	N(%)	0(0.0)	1(6.7)
FETAL INCIDENCE	N(%)	0(0.0)	7(5.0)**

(M) = MALFORMATION (V) = VARIATION
a. See the individual fetal alterations table (Appendix 11) for fetuses with skeletal alterations.
b. Dead fetuses were excluded from summarization and statistical analyses. Observations for these conceptuses are cited on Appendix 11.
** Significantly different from the control group value ($p \leq 0.01$).

9.3 Prenatal and Postnatal Development

Study title: Developmental and Peri-/Post-natal Reproduction Study of Subcutaneous rAvPAL-PEG in Rats, Including a Postnatal Behavioral/Functional Evaluation

Study no.:	BMN165-14-013
Study report location:	N/A
Conducting laboratory and location:	(b) (4)
Date of study initiation:	June 9, 2014
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	P1626-14103; 100%

Key Study Findings

1. Administration of 20 mg/kg/day reduced body weight (↓5.4-13.2%) and body weight gain (↓18.8- 25.8%) in F0 females during the periods of premating, pregnancy, and the first 10 days of lactation. Reduced body weight and body weight gain were associated with a significant decrease in food consumption (↓9.5 – 33.4%).

2. Administration of 20 mg/kg/day produced a significant increase in the number of dams (F0 females) with all pups dying during postpartum days 1 to 4, and the incidence pup deaths through postnatal day 7.

3. At 20 mg/kg/day, rAvPAL-PEG significantly reduced viability index (↓15.5%), lactation index (↓9%), survival of pups (F1) per litter from postpartum day 4 to 21 (↓20.8% - 28.7%), and pup weight/litter (↓up to 28.3%).

4. The offspring from dams treated with 20 mg/kg/day showed a significant decrease in body weight and/or body weight gain, food consumption (F1 males only), adverse clinical signs, and delayed sexual maturation.

5. Plasma rAvPAL-PEG in dams (F0 generation) increased with dose, but there was no measurable rAvPAL-PEG in plasma from pups.

6. rAvPAL-PEG levels in milk and the number of dams with measurable test-article in milk increased with dose.

7. There was a dose-dependent reduction in plasma phenylalanine in the F0 generation. The phenylalanine level was less than or slightly higher than the limit of quantification (1 µM) at 20 mg/kg/day.

8. Learning and memory in the offspring (F1 generation) were evaluated through the Passive Avoidance and M-Maze Water tests, which showed no effects of rAvPAL-PEG. However, no other tests were conducted to evaluate behavior, motor activity, sensory or sensorimotor functions, or reflex development. Therefore, this study did not provide an adequate evaluation of postnatal development.

9. The apparent NOAEL for developmental effects was 8 mg/kg/day. However, this conclusion is only preliminary, given that this study lacked sufficient testing to provide an adequate evaluation of postnatal development (see #8 above). The adverse effects in offspring at 20 mg/kg/day may have been secondary to maternal toxicity. The NOAEL for maternal toxicity was 8 mg/kg/day.

Methods

Doses:	0 (vehicle), 2, 8, 20 mg/kg/day
Frequency of dosing:	Daily
Dose volume:	2.5 ml/kg
Route of administration:	subcutaneous
Formulation/Vehicle:	10 mM TRIS, 135 mM sodium chloride, 1 mM trans-cinnamic acid, and 1 mM ammonia, pH 7.0 ± 0.5
Species/Strain:	Crl:CD(SD) female rats
Number/Sex/Group:	25
Satellite groups:	Toxicokinetics: 3
Study design:	Female rats were given the test article and/or the control article formulations via subcutaneous injection once daily beginning 28 days before cohabitation, with continuation of dosing through cohabitation, pregnancy, and Day 21 of lactation.
Deviation from study protocol:	Deviations had no impact on data integrity or interpretation of results.

Observations and Results

F_0 Dams

Survival: There were no treatment-related deaths. One high-dose female was euthanized on lactation day (LD) 20 due to adverse clinical signs (dehydration, weight loss, swollen snout, hunched posture, pale ears, tachypnea, urine-stained abdominal fur, etc.) that appeared to be related to snout injury.

Clinical signs: There were no treatment-related clinical signs.

Body weight: Females in the 20 mg/kg/day (high-dose) group had significantly reduced body weight and/or body weight gain during the periods of premating, gestation and lactation. Body weight and body weight gain in the high-dose females were reduced by 5.4% and 25.8% at the end of the premating period, respectively. At the end of pregnancy, body weight and body weight gain in the high-dose females were reduced by 10.9% and 18.8%, respectively. During the first 10 days of lactation, high-dose females had a significant decrease in body weight (↓up to 13.2%). However, females in all treatment groups had significantly increased body weight gain during lactation days 1-21 (↑1.7-, 2-, and 3.4-times the control value for the 2, 8, or 20 mg/kg/day groups, respectively).

Feed consumption: During the premating period, high-dose females had a significant reduction in food consumption (↓up to 9.5%). Females in all treatment groups had a significant decrease in food consumption during the pregnancy (↓5.5%, 5.5%, and 12.6% in the 2, 8, and 20 mg/kg/day groups, respectively). During lactation days 1 to 14, a significant decrease in food consumption (↓up to 33.4%) was observed in the high-dose females.

Uterine content: rAvPAL-PEG had no effects on the number of implantation sites, number of dams with stillborn pups, or number of dams with no live-born pups.

Necropsy observation: There were no treatment-related changes.

Toxicokinetics: Plasma samples were collected pre-dose on study days 1, 8, 15, and 22, on gestation days 14 and 21, and lactation days 7 and 14. rAvPAL-PEG levels

increased with dose. On lactation day 14, the mean values were 31,533 ± 3669, 98,633 ± 8792 ng/mL, and 553,000 ± 278,124 ng/mL for doses of 2, 8, and 20 mg/kg/day, respectively.

Dosing Solution Analysis: The concentrations of all formulations were within the acceptance criteria of ± 15% of their theoretical concentrations. Evaluations of stability and homogeneity of formulations were not conducted.

Other: There were no treatment-related effects on mating and fertility parameters (i.e. the number of days in cohabitation, females mating, fertility index, females with confirmed mating, and females pregnant/ number of female in cohabitation), duration of gestation, and gestation index.

Milk analysis: The levels of rAvPAL-PEG were measured at 2 hr post-dose on lactation day 14. rAvPAL-PEG levels in milk increased with dose level (153 ng/ml, 612 ng/ml, and 1490 ng/ml in the 2, 8, and 20 mg/kg/day groups, respectively). A dose-related increase in the number of dams with measurable test-article in milk was observed.

Plasma phenylalanine: Plasma samples were collected pre-dose on study days 1, 8, 15, and 22, gestation days 0, 14, and 21, and lactation days 7 and 14. Compared to the control values, all doses produced a dose- and duration-dependent decrease in plasma Phe levels. rAvPAL-PEG at 2 and 8 mg/kg/day reduced the Phe level from study day 15 (↓27% and↓52% at 2 and 8 mg/kg/day, respectively) through lactation day 14 (↓43% and ↓86% at 2 and 8 mg/kg/day, respectively). At 20 mg/kg/day, the Phe level was reduced to levels less than or slightly higher than the limit of quantification (1 µM) by study day 15. Phe levels were unchanged for the rest of the study. .

F_1 Generation

Survival: Pre-weaning: rAvPAL-PEG at 20 mg/kg/day significantly increased the number of dams with 100% mortality in litters during postpartum days 1-4

(3/25 dams vs. 0/25 dams in the controls). The high dose also increased the incidence of dams with total litter loss during postpartum days 5-21 (2/25 dams vs. 0 in the controls), but the increase did not reach statistical significance. At 20 mg/kg/day, rAvPAL-PEG significantly increased the number of pups found dead or presumed cannibalized on or during postpartum days 1, 2-4, and 5-7 (0.9%, 16.5%, and 9.8% compared to 0%, 2.5%, and 0.9% in the controls on days of 1, 2-4, and 5-7, respectively). Thus, 20 mg/kg/day rAvPAL-PEG significantly reduced the viability index (82.8% vs. 98% in the controls) and lactation index (89.8% vs. 98.8% in the controls). There was a significant decrease in the surviving pups per litter from postpartum days 4 to 21 (11.4% vs. 14.4% in controls on postpartum day 4; 10.2% vs. 14.3% in controls on postpartum day 21). Pups from dams treated with 20 mg/kg/day had a significant decrease in pup weight/litter during postpartum days 1 to 21 (↓up to 28.3% on day 4).

Clinical signs: Pre-weaning: rAvPAL-PEG at 20 mg/kg/day produced a significant increase in the incidence of the following clinical signs among litters and pups: cold to touch, not nursed, dehydration, not nested, and no milk in stomach (examined pups found dead and necropsied only).

Post-weaning: There were no treatment-related clinical signs.

Body weight: F1 male rats from the high-dose dams had a significant decrease in body weight during postpartum days 22 to 78 (↓17.7% on day 22 and ↓5.9% on day 78) and body weight gain during postpartum days 22 to 43 (↓up to 10.2%). Thereafter, body weight and body weight gain in males were lower than the control values, but did not reach statistical significance. F1 female rats from the high-dose dams had a significant decrease in body weight during postpartum days 22 to 29 (↓14% on day 22 and ↓9% on day 22). There was no significant change in body weight gain during postpartum days 22 to 92.

There were no treatment-related changes in body

weight or body weight gain in F1 females during pregnancy, following mating with F1 males from the same dose group.

Feed consumption: F1 male rats from the high-dose dams had a significant decrease in food consumption during postpartum days 22 to 43 (↓9.7% on days 22-29 and ↓6.4% on days 36-43). There was no significant change in food consumption in F1 females.

There were no treatment-related changes in food consumption in F1 females during pregnancy.

Physical development: The Sponsor only conducted evaluation of sexual maturation. F1 males from the high-dose dams had a significant delay in preputial separation (47.6 ± 3.5 days vs. 45.1 ± 1.8 days in the controls; $p \leq 0.01$). Vaginal opening in F1 females was also delayed (35.6 ± 2.7 days vs. 33.8 ± 2.7 days in controls; $p > 0.05$).

Neurological assessment: Learning and memory was evaluated using the passive avoidance and M-water maze tests on postnatal day 70 ± 2. rAvPal-PEG had no effect on the number of trials needed to meet the performance criteria, or latency in the passive avoidance test. No effects were observed in the performance parameters in the water maze test. No other tests to evaluate behavior, motor activity, sensory or sensorimotor functions, or reflex development were conducted. Therefore, this study did not provide a complete evaluation of postnatal development.

Reproduction: Maternal dosing with rAvPAL-PEG did not affect mating and fertility of the offspring (F1 generation).

Other: There were no treatment-related gross lesions in the F1 generation. Absolute testes weight was significantly reduced in the F1 males from the high-dose dams (↓10.2%). However, the testes weight relative to body weight was not significantly different compared to the controls.

Ovaries and Uterine content: Maternal dosing with rAvPAL-PEG had no effects on the number of copora lutea and implantations, implantation loss,

litter sizes, live/dead fetuses, early/late resorptions, sex ratio, dams with viable fetuses, or placenta in the F1 females.

Toxicokinetics: Blood samples were collected from 4 pups/litter (2 per sex) in the TK groups on lactation day 14. There was no detectable rAvPAL-PEG in plasma.

F_2 Generation

Survival: Treatment of the F0 generation had no effects on survival of the F2 generation.

Body weight: F2 fetuses in the 20 mg/kg/day group had a significant increase in total fetal weight (↑5.4%), which was due to a significant increase in male fetal weight (↑5.1%). The increase in F2 female fetal weight did not reach significance (↑4.3%).

External evaluation: Three fetuses had gross alterations. Thread like tail was observed in one fetus in each of the 2 and 20 mg/kg/day groups. One fetus from the 8 mg/kg/day group had depressed eye bulges, a small oral opening, and a misshapened snout. The malformations were not considered treatment-related since the incidence was low and there was no dose-dependent effect.

Male/Female ratio: No effects on male/female ratio were observed in F2 generation.

10 Special Toxicology Studies

Development and Validation of an Immunohistochemical Method for the Semi-Quantitative Evaluation of Anti-PEG Antibody and Anti-rAvPAL-PEG Antibody Binding in Formalin- Fixed Paraffin-Embedded Rat Tissues (Study # BMN 165-13-068)

Methods:

The objective of this study was to validate an IHC (immunohistochemistry) method by using rabbit anti-rAvPAL-PEG IgG antibody (anti-rAvPAL-PEG, 5 µg/ml) and rabbit anti-PEG (1 µg/ml). This study was conducted in February 2014.

Selected formalin-fixed paraffin-embedded (FFPE) samples (adrenal, kidney, liver, mesenteric lymph node, mandibular lymph node and spleen) from 5 vehicle control rats (0 mg/kg rAvPAL-PEG) and 5 rats treated with 25 mg/kg rAvPAL-PEG from the 26-week toxicology study with a 17-week interim sacrifice (study # BMN 165-08-019) were used to validate the IHC.

Using the validated IHC, binding of anti-rAvPAL-PEG was conducted on sections of FFPE brain, heart, lung (with large bronchi), and skeletal muscle (thigh) from the five vehicle control rats and five rats treated with 25 mg/kg rAvPAL-PEG.

Protein spots composed of either rAvPAL-PEG or purified rAvPAL bulk were used as positive control samples in all experiments. Protein spots of the control article, Bovine Serum Albumin (BSA), were used as the negative control sample.

Experiments were conducted to optimize and validate the conditions for binding of anti-PEG and anti-rAvPAL-PEG to control protein spots, and representative specimens from each selected tissue type and dosing group. The selectivity, accuracy, and precision of anti-rAvPAL-PEG binding to the validation tissues were confirmed. However, the selectivity, accuracy, and precision of anti-PEG binding were not able to be confirmed. A semi-quantitative approach to measure immunoreactivity between control and test article-treated rats using positive pixel analysis was inconclusive due to non-specific background staining in control rats.

Therefore, the IHC evaluation of study tissues was performed only with anti-rAvPAL-PEG and the data is reviewed below.

In addition, the tissue sections from the adrenal, kidney, liver, mesenteric lymph node, mandibular lymph node and spleen were stained with H&E and evaluated for tissue preservation and the presence of cytoplasmic vacuoles.

Results:

Anti-rAvPAL-PEG staining

Positive staining by anti-rAvPAL-PEG was present in renal tubular epithelium and in infiltrating or fixed phagocytes (histiocytic cells) in adrenal gland, liver, lymph node (mandibular and mesenteric), and spleen from the 5 rats treated with 25 mg/kg of rAvPAL-PEG as shown in the Sponsor's table below.

There was no positive staining by anti-rAvPAL-PEG in sections of brain, heart, lung, or skeletal muscle (thigh) samples from vehicle control or 25 mg/kg rAvPAL-PEG-treated rat samples.
There was no positive staining by anti-rAvPAL-PEG in the validation tissue samples from vehicle control animals.

Table 70: Summary of Positive Binding of Anti-rAvPAL-PEG to Validation Tissue Samples from Animals Treated with 25 mg/kg rAvPAL-PEG

Tissue	Positive Cells or Tissue Elements	Incidence	Intensity	Frequency
Adrenal	Vacuolated macrophages; serum protein and blood cells in veins, capillaries	5/5	1+ - 2+	O - C
Kidney	Serum protein and blood cells, capillaries (glomerular and peri-tubular), arteries, veins, glomerular epithelium; most intense in papilla, medulla; not in vacuoles in tubular epithelium	5/5	2+ - 3+	C - F
Liver	Vacuolated macrophages and hepatocytes; serum protein and blood cells, sinusoids, veins and arteries, rarely in Kupffer cells	5/5	1+ - 2+	C - F
Mandibular Lymph Node	Vacuolated macrophages; serum protein and blood cells, capillaries and veins	5/5	1+ - 2+	R - C
Mesenteric Lymph Node	Vacuolated macrophages; serum protein and blood cells, capillaries, subcapsular veins and lymphatics, sinusoids	5/5	1+	C - F
Spleen	Cytoplasm and vacuoles in sinusoidal macrophages, serum protein and blood cells, veins, arteries	5/5	1+	C - F

Intensity: 1+ = minimal, 2+ = mild, 3+ = moderate, 4+ = marked.
Frequency: R = Rare (<1-25% of cells of a particular cell type), O = Occasional (>25-50% of cells of a particular cell type), C = Common (>50-75% of cells of a particular cell type), F = Frequent (>75- 100% of cells of a particular cell type).

Validation of cytoplasmic vacuolation

The table below (taken from the study report) shows the comparison of findings for cytoplasmic vacuolation in tissues stained with H&E from the current study report and previous findings reported by the study pathologist in study # BMN-0165-08-019. Based on the data, the Sponsor stated the following: "*Preservation of all of the validation tissues was judged to be adequate.*"

In the adrenals, liver, mandibular lymph node, mesenteric lymph node, and spleen, the vacuolated cells were limited to macrophages (histiocytes). In the kidney, the tubular epithelium in the outer medulla contained vacuoles, but vacuolated macrophages were not observed.

The Sponsor stated the following regarding vacuolation observed in liver: "*Cytoplasmic vacuolation in hepatocytes and Ito cells was noted in the liver in all 5 liver samples evaluated. This vacuolation was consistent with the vacuolation associated with hepatic lipidosis, which is common in naive age-matched rats. This vacuolation was not recorded and was considered to be incidental.*"

Table 71: Summary of Cytoplasmic Vacuolation in Tissue Samples from Animals Treated with 0 or 25 mg/kg rAvPAL-PEG

Tissue	Cytoplasmic Vacuolation	rAvPAL-PEG, 0 mg/kg		rAvPAL-PEG, 25 mg/kg	
		0165-08-019	BMN165-13-068	0165-08-019	BMN165-13-068
Adrenal	Negative	5/5	5/5	2/5	2/5
	Minimal	0/5	0/5	3/5	3/5
Kidney	Negative	5/5	~~2/5~~ 5/5	0/5	0/5
	Minimal	0/5	~~3/5~~ 0/5	4/5	5/5
	Mild/Slight	0/5	0/5	1/5	0/5
Liver	Negative	5/5	5/5	0/5	0/5
	Minimal	0/5	0/5	2/5	3/5
	Mild/Slight	0/5	0/5	3/5	2/5
Lymph Node, Mandibular	Negative	5/5	5/5	0/5	0/5
	Minimal	0/5	0/5	0/5	1/5
	Mild/Slight	0/5	0/5	5/5	4/5
Lymph Node, Mesenteric	Negative	5/5	5/5	0/5	0/5
	Minimal	0/5	0/5	2/5	2/5
	Mild/Slight	0/5	0/5	3/5	3/5
Spleen	Negative	5/5	5/5	0/5	0/5
	Minimal	0/5	0/5	0/5	0/5
	Mild/Slight	0/5	0/5	2/5	2/5
	Moderate	0/5	0/5	3/5	3/5

Semi-Quantitative Immunohistochemical (IHC) Evaluation of Anti-rAvPAL-PEG Antibody Binding in Formalin-Fixed Paraffin-Embedded Rat Tissues (Study # MBN 165-13-069)

Methods:

This study was conducted in December 2014. Selected formalin-fixed paraffin-embedded (FFPE) samples (adrenals, kidney, liver, mesenteric lymph node, mandibular lymph node, and spleen) from vehicle control rats and rats treated with 8 mg/kg rAvPAL-PEG from the 4-week toxicity study (# 165-07-009), and the 26-week toxicology study with a 17-week interim sacrifice (study #165-08-019) were subjected to the IHC evaluation.

Kidney tissues with previously reported vacuolation from rats treated with 25 mg/kg rAvPAL-PEG in the 26-week toxicity study were used as positive control samples for

anti-rAvPAL-PEG binding. Kidney with no previously reported vacuolation from the control group in the 26-week rat toxicity study was used as a negative control in the IHC evaluation. Rabbit anti-rAvPAL-PEG IgG antibody (anti-rAvPAL-PEG) was used in the study.

Results:

Anti-rAvPAL-PEG binding

As shown in the table below (taken from the study report), the overall incidence and intensity of positive binding of anti-rAvPAL-PEG in the kidney was similar in males and females. The incidence of staining was increased with treatment duration. Positive binding was present in blood vessels (endothelium and intraluminal contents) in glomeruli, cortex, medulla and papilla. Epithelial cells in the renal papilla were positive in a few samples. Vascular staining most likely represents circulating rAvPAL-PEG within the lumen of blood vessels.

Table 72: Summary of Positive Specific Anti-rAvPAL-PEG Binding in Kidneys from Rats Receiving rAvPAL-PEG at 8 mg/kg for 4, 17, and 26 weeks

Sex	Weeks on Study	Incidence[a]	Intensity	Incidence[a] by Frequency		
				Rare	Occasional	Common
Male	4	3/10	Minimal (1+)	0/10	2/10	1/10
	17	10/10	Minimal (1+)	5/10	3/10	2/10
	26	13/15	Minimal (1+)	7/15	5/15	1/15
Female	4	7/10	Minimal (1+)	1/10	1/10	4/10
			Mild (2+)	0/10	0/10	1/10
	17	10/10	Minimal (1+)	0/10	2/10	8/10
	26	9/15	Minimal (1+)	2/15	2/15	5/15

[a] Incidence number positive at that intensity and frequency / total number evaluated.
Intensity: ± = equivocal, 1+ = minimal, 2+ = mild, 3+ = moderate, 4+ = marked, Neg = Negative, M = Missing or inadequate.
Frequency: R = Rare (<1-25% of cells of a particular cell type) , O = Occasional (>25-50% of cells of a particular cell type), C = Common (>50-75% of cells of a particular cell type), F = Frequent (>75- 100% of cells of a particular cell type).

Positive binding was also observed in blood vessels and sinusoids in 2 of 2 liver samples (from 17 and 26 weeks of treatment) and in follicular lymphocytes in 1 of 6 spleen samples (from the 4-week study). There was no specific binding of anti-rAvPAL-PEG in adrenal (n = 1), mesenteric lymph node, or mandibular lymph node.

Cytoplasmic vacuolation

Hematoxylin and eosin stained sections of all samples evaluated for binding of anti-rAvPAL-PEG were also evaluated for the presence of cytoplasmic vacuolation. Cytoplasmic vacuolation was present in tubular epithelium in the kidney and in histiocytic cells in spleen and lymph nodes from animals treated with 8 mg/kg rAvPAL-PEG for 4, 17, or 26 weeks. The incidence and severity of cytoplasmic vacuolation in kidney, spleen, and lymph nodes are summarized in the table below. No cytoplasmic vacuolation was observed in the examined organs from the control animals. The

cytoplasmic vacuoles in renal tubular epithelium, and in histiocytic cells in spleen and lymph nodes did not bind anti-rAvPAL-PEG.

The cytoplasmic vacuolation in renal tubular epithelium in animals dosed with rAvPAL-PEG at 8 mg/kg (as shown in the table below) was consistent with the finding of test article-related vacuolation/hypertrophy tubule cells in the 26-week toxicity study with a 17-week interim sacrifice (study # BMN165-08-019).

Table 73: Summary of Cytoplasmic Vacuolation in Kidney, Spleen, and Lymph Nodes from Rats Receiving rAvPAL-PEG at 8 mg/kg for 4, 17, or 26 weeks

Weeks of Dosing	Organs Examined (Vacuolation Incidence and Severity)				
	Kidney (tubular epithelium)	Spleen (histiocytic cells)		Mandibular lymph nodes (sinusoidal histiocytic cells)	Mesenteric lymph nodes (sinusoidal histiocytic cells)
	minimal	minimal	mild	Minimal	Minimal
4	0/20	4/4			
17	6/20		1/1	5/5	1/2
26	10/30	1/1		5/5	2/4

Summary findings from the study # BMN 165-13-068 and BMN 165-13-069:

Positive binding of anti-rAvPAL-PEG was present in renal tubular epithelium and histiocytic cells in adrenal gland, liver, lymph node (mandibular and mesenteric), and spleen in the 25 mg/kg group animals. At 8 mg/kg, positive binding of anti-rAvPAL-PEG was present in renal tubular epithelium (from 4, 17, and 26 week-treatment durations) and follicular lymphocytes in spleen (from 4 week treatment duration).

Cytoplasmic vacuolation in H&E stained sections was present in renal tubular epithelium, and in infiltrating and fixed phagocytes (histiocytic cells) in adrenal gland, liver, mandibular lymph node, mesenteric lymph node, and spleen, consistent with the clearance mechanisms of PEG and rAvPAL-PEG.

However, anti-rAvPAL-PEG did not bind to the cytoplasmic vacuoles in renal tubular epithelium, or to vacuolated histiocytic cells in adrenal, spleen, mandibular lymph node, and mesenteric lymph node.

There was no positive binding of anti-rAvPAL-PEG in sections of brain, heart, lung, or skeletal muscle (thigh) samples from rats treated with 25 mg/kg rAvPAL-PEG. The absence of positive binding by anti-rAvPAL-PEG in renal tubular epithelium in cortex or medulla following 8 and 25 mg/kg rAvPAL-PEG treatment for 26 weeks suggests that rAvPAL-PEG is excreted through the glomerulus with little or no accumulation in the renal tubular epithelium.

The Immunohistochemical Analysis in Rat Tissues in a 26-Week Subcutaneous Injection Toxicity and Toxicokinetic Study with a 12-Week Recovery Period and a 17-Week Interim Euthanasia with a 4-Week Recovery Period with rAvPAL PEG in Rats (study # 16-RS-357)

This immunohistochemical study report was included in the 26-week rat toxicity study (BMN 0165-08-019) and the study was conducted in 2016 using a validated IHC method.

Methods:

To evaluate PEG staining and semi-quantitatively analyze PEG accumulation, the following formalin-fixed paraffin embedded tissues from animals administered rAvPAL-PEG at doses of 0, 8 or 25 mg/kg for 17 or 26 weeks were studied: adrenal gland, kidney, spleen, liver, lymph nodes (mesenteric and mandibular), testis, and injection sites (served as control samples). The primary antibody was rabbit anti-PEG IgG or a rabbit IgG isotype control. The number of tissues examined from each group is summarized in the table below (taken from the study report).

Table 74: Number of Tissues (Test Samples) from Each Time Point† Following 17 or 26 Weeks of Treatment

	Males			Females		
Group	1*	3*	4*	1*	3*	4*
Dose Level (mg/kg)	0	8	25	0	8	25
Adrenal Gland	3	3	4[a]	3	3	3
Kidney	3	3	4[a]	3	3	3
Liver	3	3	4[a]	3	3	3
Lymph Node (Mandibular)	3	3	4[a]	3	3	3
Lymph Node (Mesenteric)	3	3	4[a]	3	3	3
Spleen	3	3	4[a]	3	3	3
Testis	6	6	7[a]	NA	NA	NA
Injection Site[b, c]	4[c]	NA	4[b]	NA	NA	NA

† Numbers of tissues (test samples) represent each time point and were doubled to yield the total number of tissues (test samples) for this study.
* Group 1 was administered control article/diluent (10 mM TRIS, 135 mM NaCl, 1 mM trans-Cinnamic Acid) only, Group 3 was administered rAvPAL-PEG (8 mg/kg), and Group 4 was administered rAvPAL-PEG (25 mg/kg).
a = An additional corresponding test sample (i.e., a serial tissue section) from one Group 4 animal was used for a negative (isotype antibody) control.
b = Injection site from Group 4 animals was used as a positive control.
c = Injection site from Group 1 animals was used as a negative control.

The anti-PEG IHC staining was assessed by examination of staining results by bright-field microscopy and semi-quantitatively scoring for staining strength (presence and intensity), staining frequency (relative density). Staining pattern (distribution) was also assessed.

Results:

As shown in the table below, samples from the 25 mg/kg rAvPAL-PEG group showed a similar anti-PEG staining, in terms of strength and frequency, at the 17- and 26-week time points. Compared to the anti-PEG staining pattern in the 25 mg/kg samples, tissues from the 8 mg/kg rAvPAL-PEG group had a weaker staining and lower frequency. Thus, there was a dose-depend effect at the examined 17- and 26-week time points (see table below).

Anti-PEG staining was predominantly observed in the vascular compartment (plasma, lymph fluid and/or on endothelial cells) of the tissue samples, suggestive of circulating rAvPAL-PEG. At 25 mg/kg rAvPAL-PEG, very limited staining of renal epithelial cells was observed at the 17-week time point, whereas this change was not apparent at the 26-week time point. Cytoplasmic anti-PEG staining was also observed in Kupffer cells in liver sinusoids, and sinus histiocytes in the mesenteric lymph nodes. The incidence, intensity, and frequency of positive binding of anti-PEG in examined tissues are summarized in the table below.

Table 75: Summary of Positive Binding of Anti-PEG in Tissue Samples from Animals Treated with 8 or 25 mg/kg rAvPAL-PEG for 17 and 26 Weeks

Tissue	Time points (week)	Dose groups (mg/kg)	Positive cells or Tissue Elements	Incidence	Intensity*	Frequency**
Adrenal	17	8	vascular spaces (endothelium and/or plasma) and sinusoids	6/6	1+ - 2+	R, O, C
		25		6/6	1+ - 2+	C, F
	26	8		5/6	1+	R, C
		25		6/6	2+	O, C, F
Kidney	17	8	vascular spaces (plasma, endothelium)	5/6	1+ - 2+	R, O, C
		25	vascular spaces (plasma, endothelium) and cortical tubules	6/6	2+ - 3+	R, O, C, F
	26	8	vascular spaces (plasma, endothelium)	6/6	1+ - 2+	O, C
		25		6/6	2+ - 3+	C, F
Liver	17	8	vascular spaces (lining sinusoids, vessels, endothelium); varied cytoplasmic staining of intra-sinusoidal cells	6/6	1+ - 2+	R, O, C
		25		6/6	2+ - 3+	F,C
	26	8		6/6	1+ - 2+	R, C
		25		6/6	2+	C, F
Spleen	17	8	endothelium and/or vascular space (plasma)	4/6	1+-2+	R, O
		25		6/6	2+ - 4+	O, C, F
	26	8		5/6	1+ - 2+	R
		25		6/6	1+ - 4+	R, O, C, F
Mandibular lymph nodes	17	8	cytoplasmic staining of predominately sinus histiocytes, lymph in sinuses, and/or vascular plasma and endothelium	6/6	1+ - 2+	R, O, C
		25		6/6	1+ - 3+	C, F
	26	8		4/6	1+ - 2+	R, O
		25		6/6	2+ - 3+	O, C

Mesenteric lymph nodes	17	8	cytoplasmic staining of predominately sinus histiocytes, lymph in sinuses, and/or vascular plasma and endothelium	6/6	1+ - 3+	O, C
		25		6/6	3+	C, F
	26	8		5/6	1+ - 2+	R, O
		25		6/6	2+ - 3+	R, C, F
Testis	17	8	vascular spaces (plasma) and endothelium	4/6	1+	R, O, C
		25		6/6	1+ - 3+	C, F
	26	8		2/4	1+	R, C
		25		6/6	1+ - 2+	R, C, F

* 1+: minimal; 2+: mild; 3+: moderate; 4+: marked

** Rare (R): <1-25% of cells of a particular cell type/tissue element; Occasional (O): >25-50% of cells of a particular cell type/ tissue element; Common (C): >50-75% of cells of a particular cell type/tissue element; Frequent (F): >75-100% of cells of a particular cell type/ tissue element

Evaluation of Ependymal Cell Vacuolation in Brains from Rats and Monkeys Treated Twice Weekly with Subcutaneous Injections of rAvPAL-PEG in Previous Chronic Toxicology Studies (study # BMN165-13-047)

Cytoplasmic vacuolation of ependymal cells was not reported by the study pathologist for either the 26-week rat toxicity study with a 17-week interim sacrifice (BMN 165-08-019) or the 39-week monkey toxicity study (BMN 165-07-030). To comply with the recommendations set forth in a white paper issued by the European Medicines Agency (EMA) on 16 November 2012 (CHMP Safety Working Party's response to the PDCO regarding the use of PEGylated drug products in the pediatric population; EMA/CHMP/SWP647258/2012), the brain sections from these two studies were reviewed by a second (reviewing) pathologist for the presence or absence of cytoplasmic vacuolation of ependymal cells, including those lining the ventricular system and the choroid plexus epithelium.

All H&E stained brain sections from the 26-week rat toxicity study and the 39-week monkey toxicity study were evaluated by light microscopy at 40x and 100x magnification for cytoplasmic vacuolation of ependymal cells lining the ventricular system of the choroid plexus. Ependymal cells in the central canal of the spinal cord were not evaluated.

Cytoplasmic vacuolation of ependymal and choroid plexus cells was not identified by the study pathologist or the reviewing pathologist in either study. Thus, twice weekly subcutaneous injections of rAvPAL-PEG in rats and monkeys did not produce vacuolation of ependymal and choroid plexus cells in the brain.

11 Integrated Summary and Safety Evaluation

Phenylketonuria (PKU) is a rare autosomal recessive disorder characterized by a deficiency in the phenylalanine hydroxylase (PAH), the enzyme that converts the amino acid phenylalanine (Phe) to tyrosine. Without functional PAH, Phe rapidly accumulates in the body, resulting in hyperphenylalaninemia (HPA). Clinical consequences of HPA are broad and significant and include severe mental retardation, microcephaly, seizures, as well as a range of cognitive, behavioral, and mood disorders (e.g., agitation, irritability, depression, schizophrenia, reduced attention span, memory deficits). Other manifestations of HPA include eczema and loss of pigmentation in skin and hair. Therefore, the primary treatment goal in the treatment and management of PKU patients is to reduce blood Phe levels to the recommended target ranges.

Palynziq (pegvaliase) is a recombinant phenylalanine ammonia lyase (rAvPAL) protein derived from the cyanobacterium *Anabaena variabilis*, which is expressed in *E. coli*. PAL catalyzes the conversion of phenylalanine to ammonia and trans-cinnamic acid. The recombinant form of PAL is PEGylated to reduce immunogenicity.

The proposed indication for pegvaliase is to reduce blood phenylalanine (Phe) levels in adult patients with PKU who have uncontrolled blood phenylalanine levels > 600 µmol/L on existing management. The recommended maximum dose is 40 mg per day via SC injection.

To support the intended clinical use, the Sponsor conducted studies in pharmacodynamics (PD), safety pharmacology, general toxicology, and developmental and reproductive toxicology in mice, rats, monkeys, and rabbits. The PD studies were conducted in BTBRPahenu2 (ENU2) mice, an animal model of PKU.

Pegvaliase at subcutaneous doses of 0.2 to 120 mg/kg given once weekly or three times per week in ENU2 mice produced a dose- and dosing frequency-dependent decrease in blood Phe levels associated with increased weight gain, lifespan, and reproductive capabilities. Pegvaliase at 10 to 20 mg/kg/week for 12 weeks increased the number of tyrosine hydroxylase-expressing neurons in hypothalamus, midbrain, and dorsomedial hypothalamic nucleus, although the number of neurons was still lower than that of wild type mice. An interim attenuated PD response due to anti-pegvaliase antibody formation was usually seen between weeks 3-7 of treatment. Increasing anti-pegvaliase titers were observed throughout the repeat dose studies.
A single SC dose up to 125 mg/kg pegvaliase had no effects on CNS or respiratory functions in rats. No cardiovascular effects were observed at a single dose of 10 mg/kg in monkeys.

After a single SC administration in rats and monkeys, first-order absorption and monophasic elimination were observed, with no apparent sex-related differences observed. The bioavailability of pegvaliase after SC injection in the rat was low (13% to 17%). Following administration of repeated doses via twice weekly SC injection in the two species, plasma drug concentrations increased after week 1 in rats and week 2

monkeys. Thereafter, pegvaliase levels were decreased, likely due to the formation of neutralizing or eliminating antibodies against pegvaliase. Plasma levels of pegvaliase increased in a dose-dependent manner with little to no accumulation. The T_{max} for the rat and monkey with single SC injection ranged from 12 to 120 hours and 48 to 108 hours, respectively, indicating a slow rate of absorption from the injection site into circulation. The apparent terminal half-life ($t_{1/2}$) ranged from 33 to 53 hours and 51 to 92 hours in rats and monkeys, respectively, demonstrating a slow rate of elimination. The long terminal $t_{1/2}$ observed in the rat with SC dosing was consistent with the long half-life observed with IV dosing (25 to 46 hours).

In a 4-week SC toxicity study, SD rats were treated with pegvaliase at doses of 1, 8, and 25 mg/kg twice weekly. Pegvaliase had no adverse effects on body weight, food consumption, hematology, clinical chemistry, or urinalysis. Pegvaliase at ≥ 8 mg/kg produced minimal vacuolation in reticuloendothelial cells in spleen and fibrosis at the injection site. None of these findings were considered adverse. Therefore, the NOAEL was 25 mg/kg. Overall, there was a dose proportional increase in systemic exposure to pegvaliase. No accumulation of pegvaliase was observed following repeated doses. Anti-drug antibodies (IgG) was detected in some animals, and the incidence and titers were not dose-dependent.

In the 26-week SC toxicity studies with a 17-week interim sacrifice, SD rats were treated with pegvaliase at doses of 1, 8, or 25 mg/kg/day twice weekly. Slight to moderate decreases in body weight, body weight gain, and food consumption were observed in the 25 mg/kg group. Pegvaliase at 8 and/or 25 mg/kg produced a slight to moderate decreases in triglycerides, ALT, ALP, and urine pH. Following the 17- and 26-week treatment periods, similar treatment-related histopathological changes were observed in the kidney, spleen, liver, testes, adrenal gland, mesenteric and mandibular lymph nodes, and subcutaneous injection sites in the 8 and/or 25 mg/kg groups. The histopathological changes included focal to multifocal areas of vacuolation/hypertrophy of renal tubule cells, increased vacuolation in histiocytic cells in the adrenal cortex, liver, spleen, mesenteric and mandibular lymph node, and testes. The incidence and severity of these changes increased with dose level and treatment duration. At the end of the 12-week recovery phase, all of these changes persisted, but with less severity. Anti-pegvaliase antibodies did not bind to the cytoplasmic vacuoles in renal tubular epithelium and in histiocytic cells in spleen and lymph nodes. However, PEG was detected (via IHC using anti-PEG antibodies) in Kupffer cells in liver sinusoids, sinus histiocytes in the mesenteric lymph nodes, and in renal epithelial cells. Kidney was considered as the target organ of toxicity, based on the incidence of vacuolation/ hypertrophy of renal tubule cells at ≥ 8 mg/kg, which failed to reverse after the 12-week recovery period. The NOAEL was 1 mg/kg twice weekly. Systemic exposure to pegvaliase generally increased in a dose-proportional manner. No accumulation of pegvaliase occurred following repeated doses. In general, females had higher pegvaliase C_{max} and AUC_{0-t} values than males. Anti-drug antibodies (IgG) were detected in most animals. The vacuolation observed in multiple organs was only associated with slight to moderate decreases in liver enzymes (AST, ALP) and urine pH. Thus, the clinical importance or relevance is unclear.

In the 4 week SC toxicity study, monkeys were treated with pegvaliase at doses of 0.01, 0.1, or 1 mg/kg twice weekly. Pegvaliase at ≥ 0.1 mg/kg caused minimal to slight vascular degeneration, mainly in the medium-sized, muscular arteries of the following organs: lung, stomach, gallbladder, kidney, colon, pancreas, spleen, and prostate. The vascular degeneration was reversible. No IHC (immunohistochemistry) was conducted to investigate whether the vascular degeneration was related to immune complexes. The target organs of toxicity were arteries and injection sites. The NOAEL was 0.01 mg/kg based on degeneration of arteries at 0.1 mg/kg and higher.

In the 39-week SC toxicity study, monkeys were treated with pegvaliase at doses of 0.01, 0.1, 3, or 7/5/3 mg/kg twice weekly. The high dose of 7 or 5 mg/kg was not tolerated based on the severe reduction in food consumption, significant body weight loss and/or hypoactivity. Thus, the dose was reduced to 3 mg/kg. Pegvaliase at ≥ 3 mg/kg produced systemic arteritis involving small arteries and arterioles in a wide range of organs and tissues (kidney, urinary bladder, pancreas, gallbladder, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, lung, heart, sciatic nerve, lacrimal gland, mandibular lymph node, epididymis, seminal vesicle, ovary, uterus, cervix, and vagina) and in subcutaneous injection sites. No arterial inflammation was observed at the end of the recovery period. Immune-complex material was detected in affected vessels in the organs examined (heart, kidney, and liver) in animals treated at ≥ 3 mg/kg. Pegvaliase and/or its PEG moiety identified by IHC were detected in intravascular proteinaceous material, endothelium, interstitial proteinaceous material, and mononuclear cells in the liver and kidney at ≥ 3 mg/kg. There were no treatment-related changes in hematology or clinical chemistry. The target organs of toxicity were arteries in multiple organs and bone marrow (increased lymphoid nodules). The NOAEL was considered to be 1 mg/kg twice weekly. The drug-induced systemic arteritis was likely related to the vascular accumulation of immune-complex material generated from the immunogenic response to pegvaliase, since immune-complex was detected in the affected vessels. Systemic arteritis was not associated with adverse changes in hematology, clinical chemistry, or histopathology in liver, kidney or other organs. Arterial inflammation was reversible after cessation of treatment. Thus, the clinical importance and translatability to humans of systemic arteritis observed in the monkeys is unknown.

In a combined fertility and embryo-fetal development (segment 1/2) study, male and female rats were treated with pegvaliase at doses of 0, 2, 8 or 20 mg/kg/day via SC injection before cohabitation, with continuation of dosing through mating, implantation and closure of the hard palate GD 17. At 20 mg/kg/day, pegvaliase produced maternal or paternal toxicities (e.g. slight to moderate decreases in body weight, body weight gain, and food consumption). Pegvaliase at ≥ 8 mg/kg/day significantly reduced the number of corpora lutea and implantations, litter size (20 mg/kg/day only), live fetuses (20 mg/kg/day only), and fetal weights (20 mg/kg/day only). Pegvaliase at ≥ 8 mg/kg/day significantly increased fetuses with alterations, which were limited to variations such as cervical ribs, bifid centra of lumbar and thoracic vertebrae, and incomplete ossification of squamosal bones, frontal bones, lumbar vertebra arch, and ribs. Systemic exposure to pegvaliase was detected in fetuses from the 20 mg/kg

group. Pegvaliase produced a dose-dependent decrease in blood Phe levels in both sexes. The Phe level was below the low limit of quantification (1 µM) at 20 mg/kg/day. The NOAEL for male fertility was 20 mg/kg/day, and the NOAEL for female fertility was 8 mg/kg/day based on the decrease in corpora lutea at 20 mg/kg/day. The NOAEL for embryo-fetal development was 8 mg/kg/day, based on the reduced fetal weights at 20 mg/kg/day.

In an embryo-fetal development study in rabbits, pregnant females were treated with pegvaliase at doses of 2 or 5 mg/kg daily (SC) using a divided dosing regimen (GD 7 to 12, GD 11 to 16, or GD 15 to 20), to provide the most consistent level of plasma exposure to pegvaliase throughout organogenesis (GD 7 to 20). The rationale for this study design was supported by toxicokinetic data from the dose-ranging study in pregnant rabbits, which demonstrated profound changes in AUC and C_{max} after a few days of dosing (values were decreased with 2 mg/kg/day, and increased with 5 mg/kg/day). The changes in TK parameters with repeated dosing were likely due to the effect of anti-drug antibodies. Subcutaneous administration of 5 mg/kg/day produced a high incidence of external malformations of the head, body, and limbs, and multiple malformations in visceral organs and all regions of the skeletal system (e.g. > 50% incidence of shortened limbs among fetuses and litters). Although the dose which produced malformations also caused clear signs of maternal toxicity (e.g. abortion and premature death in 8% of rabbits, marked impairment of weight gain and food consumption in the surviving females), the malformations are not considered to be secondary to maternal toxicity based on their severity and high incidence. Other adverse effects observed at 5 mg/mg/day included significant increases in late resorptions, post-implantation loss, and the number of does with any resorptions. Reductions in male and female fetal weight and the number live male fetuses were also observed. The observed reduction in maternal weight gain during days 7-29 of gestation was 23% and 59% in the 2 and 5 mg/kg/day groups, respectively. These values represent the mean reduction observed in the three treatment periods used in this study (i.e. GD 7-12, 11-16 and 15-20). During treatment (days 7-20 of gestation), plasma Phe levels in the 2 and 5 mg/kg/day groups were decreased by an average of 90% and 99%, respectively. The NOAEL for embryo-fetal developmental toxicity was 2 mg/kg/day. The maternal NOAEL was not identified (< 2 mg/kg/day), due to the reductions in weight gain and food consumption in the 2 mg/kg/day group.

In a pre-/postnatal development study in rats, pegvaliase at 20 mg/kg/day produced significant decreases in maternal body weight gain (up to 25.8%) and food consumption (↓up to 33.4%). At 20 mg/kg/day, pegvaliase significantly reduced viability index (↓15.5%), lactation index (↓9%), pup survival during postpartum days 1-4 (↓up to 28.7%), and pup weight during lactation (↓up to 28.3%). The offspring from the 20 mg/kg/day group had significant decreases in body weight, body weight gain, and food consumption (males only), and showed a delay in sexual maturation. No pegvaliase was detected in the offspring. However, pegvaliase was detected in milk at all doses (2, 8, and 20 mg/kg/day). There was a dose-dependent reduction in maternal plasma phenylalanine level. The apparent NOAEL for developmental effects was 8 mg/kg/day. However, this conclusion is only preliminary, given that this study lacked sufficient

testing to provide an adequate evaluation of postnatal development. Learning and memory in the offspring (F1 generation) were evaluated through the Passive Avoidance and M-Maze Water Maze tests, which showed no effects of pegvaliase. However, no other tests were conducted to evaluate behavior, motor activity, sensory or sensorimotor functions, or reflex development. The adverse effects in offspring at 20 mg/kg/day may have been secondary to maternal toxicity. The NOAEL for maternal toxicity was 8 mg/kg/day. This study should be repeated with the inclusion of tests to evaluate the effects on behavior, motor activity, sensory or sensorimotor functions, and reflex development.

Vacuolation in multiple organs in the repeat-dose rat toxicity studies (4, 17 and 26 weeks) at ≥ 8 mg/kg was further evaluated in selected organs (adrenal, kidney, liver, mesenteric lymph node, mandibular lymph node, spleen, and testis) by a validated IHC method using anti-pegvaliase and anti-PEG antibodies.

Minimal to mild binding of anti-pegvaliase antibodies was present in kidney, and the incidence and severity was related to dose and duration of treatment. Binding of anti-pegvaliase antibodies in blood vessels (endothelium and intraluminal contents) was observed in glomeruli and in interstitial vessels in cortex, medulla, and papilla of kidney. The vascular staining most likely represented circulating pegvaliase within the lumen of blood vessels. Binding in epithelial cells was cytoplasmic and present only in the renal papilla in some animals. Binding was present in blood vessels and sinusoids in liver (weeks 17 and 26) and in follicular lymphocytes in some spleen samples (week 4). Anti-pegvaliase antibodies did not bind to vacuoles in renal tubular epithelium, histiocytic cells in spleen, or in mandibular and mesenteric lymph nodes. Cytoplasmic anti-PEG staining was mainly present in vascular spaces (plasma/endothelium) in kidney, adrenal gland, spleen, liver, lymph node (mesenteric mandibular) and testes. Cytoplasmic anti-PEG staining was observed occasionally in Kupffer cells in liver sinusoids, sinus histiocytes (lymph node), or renal tubular cells (only in rats administered 25 mg/kg through study week 17). The intensity of staining was dose-dependent.

The presence of positive staining by anti-pegvaliase antibodies in renal tubular epithelium and in infiltrating or fixed macrophages (histiocytes) in adrenal gland, liver, lymph node (mandibular and mesenteric), and spleen was likely indicative of the clearance mechanisms for PEG.

Leachables, including organic and elemental leachables, were detected in the drug product (pre-filled syringe) stored at 2-8°C for 24 months or at 25°C for 6 months (accelerated conditions). The toxicology risks of these leachables and elements were assessed. The potential exposure to leachables at the maximum recommended daily dose of 40 mg pegvaliase does not present any safety concerns (see section 2.5 and Appendix for details).

12 Appendix/Attachments

12.1 Toxicological risk assessment for leachables and extractables from prefilled syringe (PFS)

The to-be-marketed container closure system for pegvaliase is a single-use 1 mL long (b) (4) glass prefilled syringe (PFS) with staked needle. The plunger stopper is composed of (b) (4) rubber (b) (4) (b) (4).

The primary packaging components include the following: (b) (4) prefilled syringe (PFS) barrel, stainless steel needle, (b) (4) rigid needle shield (RNS) rubber formulation, and (b) (4) plunger stopper. For the leachables study, the pegvaliase samples were stored at 2-8°C for 24 months or at 25°C for 6 months (accelerated conditions).

The samples from the preliminary and final leachables studies were analyzed based on the characteristics of extractables and leachables using the following methods:

A. Gas chromatography mass spectrometry (GC/MS) was used (b) (4) (b) (4) and headspace GC/MS was used (b) (4) (b) (4)).

B. The leachables samples were also analyzed by liquid chromatography with ultraviolet and mass spectrometry detection (LC/UV/MS) (b) (4) (b) (4); retention times and spectra were compared to a database comprised of standards.

C. Elemental analysis was performed using inductively-coupled plasma optical emission spectroscopy (ICP-OES) and ICP-MS was used for the simulation and leachables studies, respectively.

D. ICP-OES was also used to measure the amount of leached (b) (4).

E. Tentative identifications in the GC/MS analyses were made possible using the Wiley2010/NIST2011 database.

For each assay, a control sample (PFS with drug product without subjecting to simulation or leachable procedures) was prepared and analyzed in the same manner as the sample to be analyzed to eliminate the potential contamination from the labware or reagents that could be misinterpreted as a extractable or leachable compound.

1. Preliminary Leachables Study

Prior to conducting a formal leachables study, a preliminary study was performed to identify and qualify extractables as potential leachables under stressed storage conditions. In the simulation study (study #: NS-07329408), PFS samples with rAvPAL-PEG DP at 20 mg/mL (20 mg dose) were incubated horizontally at 60 °C for 30 days prior to subsequent sample preparation for analysis.

No metal elements were detected in the extracts (detection limit of (b) (4) µg/ml) by ICP-OES. In the ICP-OES analysis, (b) (4) or (b) (4) was detected in DP, a concentration of (b) (4) µg/mL. The reporting limit for (b) (4) was (b) (4) µg/ml.

2. Final Leachables Study

In the final leachables study (study #: NS-08403016), the 2.5 mg dose (5 mg/ml) and 20 mg dose (20 mg/ml) PFS samples were stored at 5 ± 3 °C up to 24 months or at the accelerated temperature of 25°C and 60% relative humidity (RH) for up to 6 months prior to subsequent sample preparation for analysis. Samples were collected prior to storage and at 0, 1, 3, 6, 9, 12, 18, and 24 months following storage. In the headspace GC/MS analysis, (b) (4) at concentrations up to (b) (4) µg/ml and (b) (4) at concentrations up to (b) (4) µg/ml were reported for all time points for the 20 mg/mL drug formulation samples, except for the 24 month time point. The 24-month sample stored at 5°C was from a different lot filled in the same fill-finish site. No compounds were reportable in any of the time points for the 5 mg/mL drug formulation. The reporting limits for (b) (4) and (b) (4) were a signal-to noise ratio greater than or equal to (b) (4)

3. Toxicological risk assessment for leachables and extractables from PFS

As shown in the table below, (b) (4) ((b) (4) ((b) (4) µg/ml), (b) (4) ((b) (4) µg/ml), and (b) (4) ((b) (4) µg/ml) were detected in the preliminary and/or final leachables study. The proposed maximum dose for rAvPAL-PEG is 40 mg per day SC in a dose volume of 2 ml (20 mg/ml). The estimated maximum exposure to the detected leachables is presented in the table below.

Table 76: Maximum Daily Dose of Leachables from PFS via SC Administration of Pegvaliase

Identification	Estimated maximum concentration (µg/ml) in DP	Maximum dose of DP(ml)	Maximum daily dose of leachables (µg per day)
(b) (4)		(b) (4)	
(b) (4)			

*The recommended maximum daily dose of rAvPAL-PEG in humans is 40 mg (20 mg/ml x 2 = 40 mg).

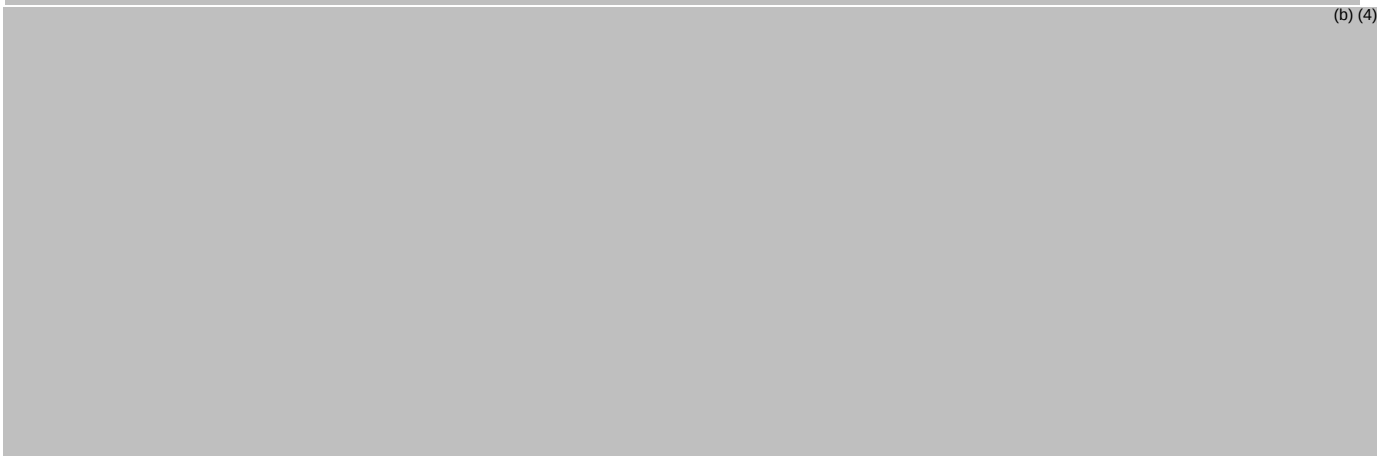
In general, the safety assessment of leachables and extractables considered as genotoxic or potentially genotoxic, or those to known to be genotoxic carcinogens will be based on compliance with the acceptable intake as recommended in ICH guidance M7. For leachables and extractables that are non-genotoxic, a daily intake of up to 5 µg is acceptable without further qualification, based on recommendations of the PQRI (Safety Thresholds and Best Practices for Extractables and Leachables in Orally Inhaled and Nasal Drug Products, September 2006). Exposure to non-genotoxic leachables with known irritant and/or sensitizing potential is acceptable at amounts up to 5 µg/day. In standard practice, if the maximum potential exposure for a non-genotoxic, non-irritant/non-sensitizing leachable or extractable exceeds 5 µg/day, compliance with the PDE (Permitted Daily Exposure) for the chemical, as calculated based on the formulas shown in ICH guidance Q3C(R6) and Q3D, can be used for safety assessment when other established limits for intake from health authorities are not available. The PDE is a safety metric that represents the maximum daily dose of a chemical considered as safe in humans, and is routinely expressed as the total daily dose (e.g. µg/day) in adults, rather than a weight based dose. Therefore, the PDE values used for safety assessment in this review are expressed as the total daily dose based on the assumed bodyweight of 50 kg in adults.

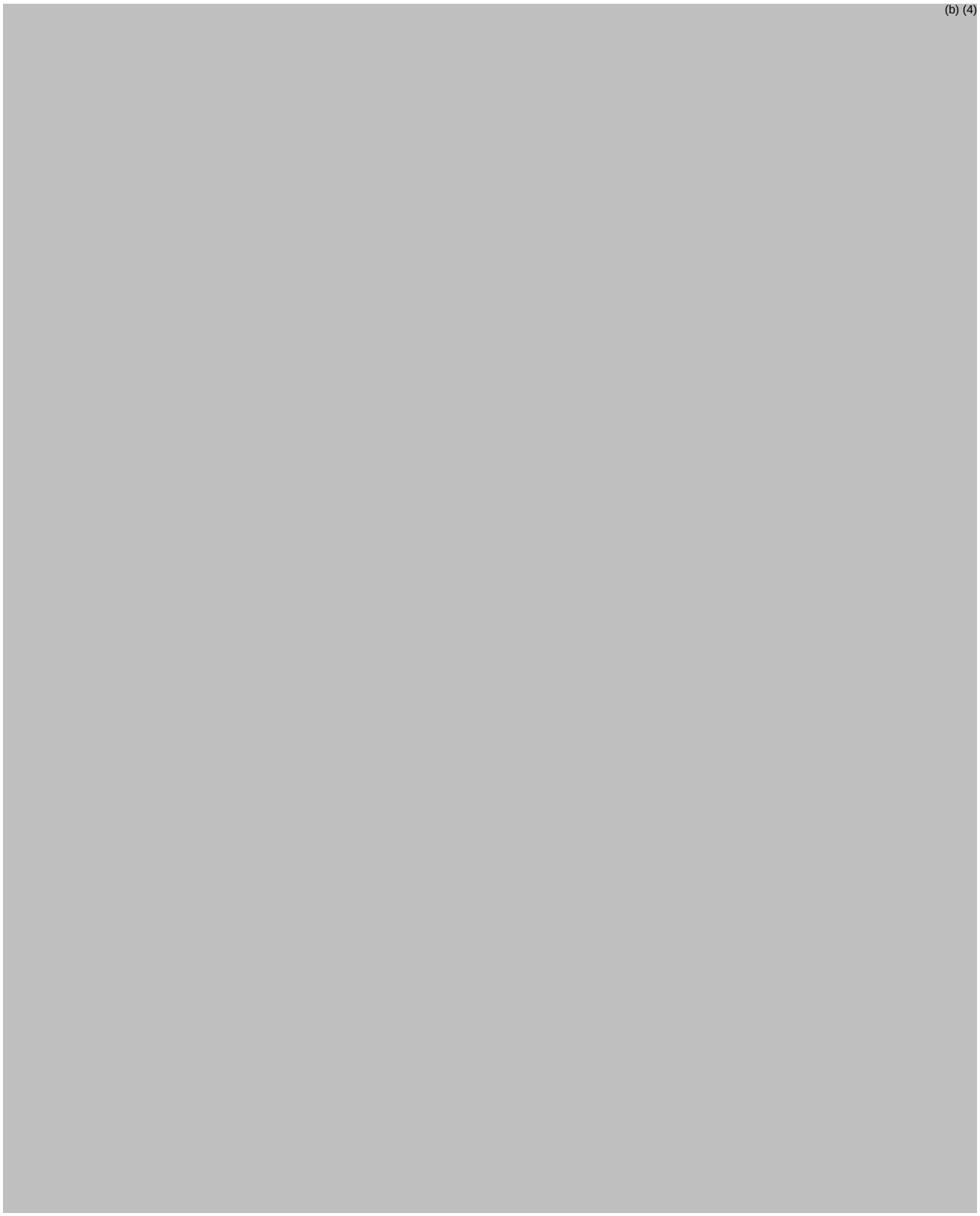
The PDE is calculated based on the following formula, which is similar to the PDE formula in ICH guidance Q3C(R6) (Impurities: Guideline for Residual Solvents):
PDE = NOEL* or NOAEL** ÷ (F1 x F2 x F3 x F4 x F5 x 10)

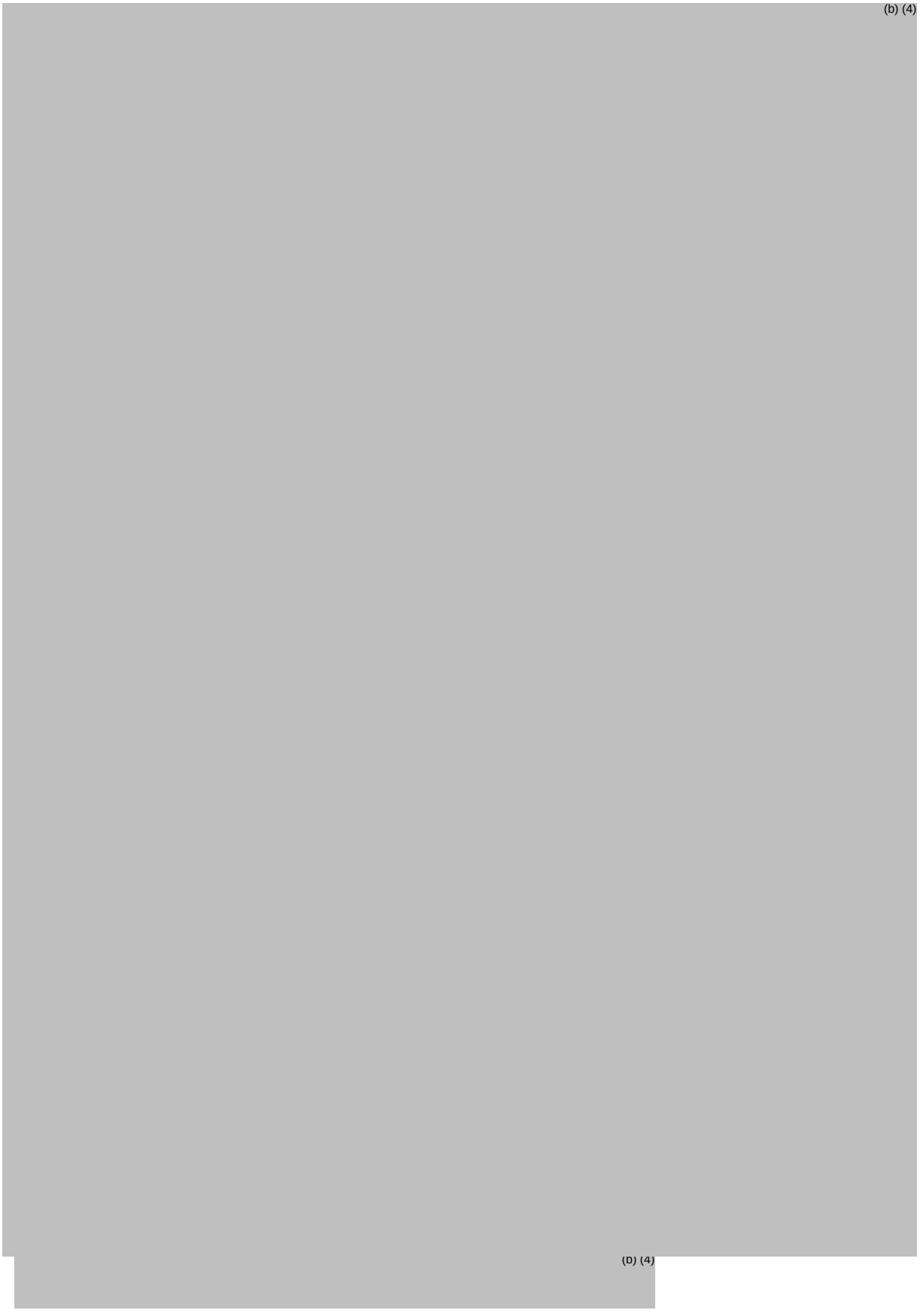
F1: A factor to account for extrapolation between species
F2: A factor of 10 to account for variability between individuals
F3: A variable factor to account for toxicity studies of short-term exposure
F4: A factor that may be applied in cases of severe toxicity (e.g. nongenotoxic carcinogenicity, neurotoxicity or teratogenicity)
F5: A variable factor that may be applied if the NOEL was not established
F6=10: Safety factor for oral to subcutaneous conversion for oral toxicity studies
* NOEL: No observed effect level
** NOAEL: No observed adverse effect level

The toxicological risk assessments from the Sponsor were provided in the format of memorandums. However, the memorandums did not include information about the supporting publications used to provide scientific evidence to support the risk assessment. An information request was sent to the Sponsor on December 8, 2017 to request these publications. On January 23, 2018, the Sponsor submitted a list of these publications, but did not identify the statements in the risk assessment memorandums that were supported by these publications. Therefore, in the review below, the references cited in the review are provided by this reviewer's own literature search.


(b) (4)


(b) (4)


(b) (4)



(b) (4)

Elemental Impurities

A total of 34 elements, including (b) (4) for which safety assessment is recommended in ICH guidance Q3D, were detected in the leachables and extraction studies using a non-validated ICP/MS. The reporting limits were (b) (4) ng/ml.

The concentrations of elements that are designated as class 1, 2, or 3 ((b) (4) (b) (4)) in the ICH Q3D were less than (b) (4) ng/mL, except for (b) (4) ng/ml). Based on the recommended maximum daily dose of 40 mg pegvaliase, delivered in 2 ml of drug product containing 20 mg/mL, the estimated maximum daily intake for most of these elements will be less than (b) (4) µg ((b) (4) µg for (b) (4)). Therefore, the worst case exposure to these elements, including (b) (4), will not exceed the respective parenteral PDEs stated in ICH Q3D. Thus, there is no safety concern about the potential exposure to these elements with administration of the maximum daily dose of pegvaliase.

The elements with no class designation or PDE stated in ICH Q3D that were present at concentrations higher the (b) (4) ng/mL are listed in the table below. The estimated maximum exposure to (b) (4) or (b) (4) is (b) (4) µg/day for each element. The element with the highest potential exposure is (b) (4) for which maximum potential dose (b) (4) µg/day) is equal to as the lowest parenteral PDE among all elements in ICH Q3D (i.e. (b) (4) µg/day for (b) (4)). Therefore, the risk appears to be low, and there is no safety con ern about the potential exposure to these elements with administration of the maximum daily dose of pegvaliase.

Table 78: Maximum daily exposure to elements with no class designated in ICH Q3D

Elements	Reported Level (ng/mL)	Maximum Daily Exposure (µg/day)	Parenteral PDE (µg/day)*
(b) (4)	(b) (4)		N/A
			N/A
			N/A
			N/A
			N/A
			N/A
			N/A

*PDE values for the listed elements are not provided in ICH Q3D

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

FANG CAI
03/06/2018

DAVID B JOSEPH
03/06/2018
I concur.